# Therapeutic Approaches To Genetic Disorders

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**By**

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## Aim of the work

The present work aims at presenting, as complete and updated as possible, a review of current approaches of treatment and management of genetic disorders. It also aims at offering a review of prospective treatment strategies for many genetic disorders for which no current effective or safe therapy is available.

Due to the very wide spectrum of these disorders regarding their diverse underlying etiologies, different pathogenic and pathogenetic mechanisms, and varied complications and prognostic consequences, the list of possible and potential therapeutic and/or alleviating measures to lessen the sufferings of patients with these diseases is seemingly endless in view of the availability of a similar endless list of different treatment and management approaches to human diseases in general.

## Scheme of the work

The ever expanding number of genetic diseases makes any attempt to outline available and expected therapies of these diseases quite difficult, not only because of their very large number but also due to the very wide and bizarre spectrum of the underlying causative mechanisms and known clinical phenotypes. In such a situation, defining therapeutic approaches to genetic diseases might be attained via revealing such treatment(s) for each disease, which seems practically impossible; through defining specific therapeutic modalities and listing together diseases amenable to treatment by this modality, which is a tedious task with many practical disadvantages; or by classifying the diseases into distinct clinical-pathological categories and outlining the different treatment(s) available for each category. Actually, none of these methods is satisfactory because of the drawbacks of each. Accordingly, we resorted to a scheme combining the three methods in preparing this work. We began by defining a general overview of currently available therapeutic modalities for genetic diseases, then we tried to outline the application of each of these modalities in treatment of the major and most common clinical-pathological categories of these diseases. For some new treatment techniques, we just listed expected diseases that might fit to be candidates for trial with these techniques. We know that this is, and might, not be the best way to handle the subject but we suppose that, and hopefully wish, it might be the more practical and useful approach in this respect.

## Introduction and general overview

There is a very wide spectrum of pathogenetic mechanisms that underlie the development of genetic diseases. These mechanisms affect either the integrity or the harmony of the strict genomic framework that controls and regulates the functions of a countless number of metabolic networks, each in turn consisting of a variable number of metabolic circuits that mediate all aspects of cell activities. This regulation is attained through the cooperative and complementary actions of the many thousands of genes comprising the genome. The scope of effects exerted by these pathogenetic mechanisms starts by disrupting gene structure via mutation and ends by synthesis of a deficient or defective gene product. The absent or insufficient activities of these gene products trigger a cascade of pathophysiological alterations leading ultimately to pathogenesis and development of the genetic disorder.

The vast majority of genetic defects develop according to the previous scenario, and are attributed to deficient or defective production of structural proteins, of regulatory proteins, or of catalytic enzymes which are the gene products of **structural genes**. Albeit, a considerable proportion of genetic diseases results from defective regulation of functions of structural genes, which is a major task exerted by **regulatory genes**. This defective regulation can happen at any point and can affect any stage of gene function and comprises a quite large spectrum of variable pathogenetic mechanisms. Examples of these mechanisms include disturbed chromatin remodeling, deficient or defective synthesis and or functions of micro- or small- RNAs, defective regulation of mRNA processing, splicing and turn over, defective monitoring of post-translation modifications due to e.g. defective chaperon actions, defective regulation of cell cycle dynamics that commonly results in development of tumors, defective regulation of morphogenetic mediators with consequent diversion towards teratogenesis and development of congenital malformations. The list is too long and includes a wide variety of different mechanisms all of which result in pathogenesis and development of a large number of genetic disorders due to defective regulatory functions exerted by regulatory genes over structural genes (**James Watson et al, 2004**).

In view of the aforementioned simplified scheme of gene function, the list of therapeutic approaches to genetic disorders seems endless, at least theoretically. Currently, there are too many therapeutic approaches to genetic diseases targeting either the underlying pathogenetic mechanism(s) or aiming at reducing the side effects and deleterious consequences of these diseases secondary to altered pathophysiological states (Peter and Sian Ellard, 2007). These therapeutic approaches include a wide variety of options including **dietary management** of inborn errors of metabolism through restriction of offending substrate (e.g. phenylketonuria, maple syrup urine disease, tyrosinemia type I) and removal of toxic products (Benson, and Fenson, 1985), **drug therapy** for e.g. hypercholesterolemia, congenital adrenal hyperplasia, and convulsive disorders (Nyhan and Ozand, 1998), **replacement therapy** of abnormal or deficient proteins or enzymes (e.g. hemophilia and many storage disorders), **cell-tissue-organ transplantation** (e.g. for many disorders like tyrosinemia and polycystic kidney disease), **surgical intervention** for genetically-determined congenital malformations and late developing surgically

correctable complications, and **gene therapy approaches** that comprise innumerable varieties of techniques aiming at correcting molecular defects leading to genetic diseases at nearly all known pathogenetic targets of these defects (e.g. exon skipping for Duchenne muscular dystrophy) (**van Deutekom et al., 2007**).

The Choice of the best therapeutic approaches to a certain genetic disorder is determined mostly by both the underlying pathophysiological mechanism(s) and the resulting deleterious complications of the defect. For instance, genetic metabolic errors caused by deficient and or defective production of enzymes necessary for metabolism of ingested food substrates (proteins, carbohydrates, lipids and other nutrients) are characterized in general by accumulation of the ingested stuff in blood. In most cases, metabolic by-products of the accumulating substrate also attain high levels secondary to the metabolic block. Percolation of the accumulating substances to cells, tissues and organs follows with subsequent pathogenesis of toxic effects exerted on cells and tissues not acquainted to the presence of these substances in such high concentrations, hence the development of clinical manifestations and disease complications, e.g. neurotoxicity exerted by hyperphenylalanenemia in PKU and by hyperammonemia in urea cycle defects, cataract formation due to accumulation of the the sugar alcohol galactitol in galactosemia, and the multi-organ toxicity observed in tyrosinemia type I due to the effects of the mitochondrial toxin succinylacetone which is a decarboxylation product of succinyl acetoacetate, a compound derived from the tyrosine catabolic intermediate fumarylacetoacetate. fumarylacetoacetate itself induces mitotic abnormalities and instability in the genome (**Jorquera and Tanguay, 2001**). Taken together, these data form the basis for a unifying hypothesis regarding the development of hepatocellular carcinoma in children with hereditary tyrosinemia.

In addition to the accumulation of the ingested stuff and its metabolic intermediates and/or its final metabolites, **deficiency of the products**, produced in normal amounts in normal conditions, results with consequent deleterious complications determined by the missing physiological and metabolic functions of these products. For instance, young women suffering from PKU give birth to very severely malformed children, a condition known as PKU embryopathy that comprises microcephaly, mental retardation, hypotrophy and cardiopathy, unless they strictly take up the specific diet, until the PHE level has lowered down to normal, before the beginning of gestation. Serotonin **deficiency**, which is a feature of PKU, probably underlies the development of PKU embryopathy in view of its morphogenetic role in normal embryogenesis (**Ch. Roux et al, 1995**).

**Substrate restriction / removal approach**

Therapeutic approaches to most genetic inborn errors of metabolism include a comprehensive handling of all aspects of the metabolic defects and include **dietary restriction of intake or removal of the offending substrate from diet** e.g. restriction of protein intake in PKU and urea cycle defects and removal of galactose and fructose from diets of infants affected with galactosemia and fructosemia, respectively.

**Removal, degradation or detoxification of the accumulating toxic metabolites** represents an indispensable and integral part of particularly in management of acute decompensation states of metabolic intoxication errors as well as in similar genetic diseases due to storage of ingested food constituents or storage of metabolic intermediates or final metabolites. Examples of this approach include use of sodium benzoate or sodium phenylbutyrate, oral lactulose, and hemodialysis / peritoneal dialysis in states of hyperammonemia, use of plasmapheresis in Refsum disease, use of iron chelation in thalassemias, and many other conditions.

**Substrate addition / replacement approach**

**Supplementation of deficient gene product(s)** represents the most proper therapeutic approaches for genetic diseases due to mutations resulting in deficient or defective production, or complete absence, of the gene products whether these are proteins, enzymes or other effector components. This approach is the oldest conventional approach adopted for this category of genetic defects and is still in practice. Treatment of hemophilia with anti-hemophilic globulin, treatment of hypothyroidism with L-Troxine, treatment of short stature due to growth hormone deficiency with GH and treatment of diabetes mellitus with insulin are just few examples.

**Supplementation of the deficient or defective enzyme** seems the logical and effective physiological therapeutic approach for inborn errors due to deficient production of the enzyme, its production in a defective configuration or failure in targeting the synthesized enzyme from the cytosol to its final destination, e.g. the lysosome or the peroxisomes, as is the case in lysosomal storage disorders and peroxisomal storage defects, respectively. Though supplementation of deficient proteins seems a straightforward approach, supplementation of enzymes is a little bit more complicated due to the intra-cellular and intra-organellar localization of most enzymes.

**Enzyme replacement therapy (ERT)** is a therapeutic approach in which the specific enzyme that is inactive or absent in affected individuals is replaced with synthetic functional enzyme preparation. ERT has been successful for the treatment of Type 1 Gaucher disease, Fabry disease, Hurler disease and, most recently, has received approval for Pompe disease. ERT is also effective in the non-neurological symptoms of Mucopolysaccharidosis Types I, II IV and VI, Pompe and Niemann-Pick B, but has not yet proven to be beneficial in storage diseases that

primarily affect the central nervous system because the replacement enzymes do not efficiently cross the blood-brain barrier.

However, the invention of recombinant enzyme, and protein, synthesis technology by genetic engineering has made a revolutionary breakthrough in genetic therapies and marked achievements have been done in this respect, and the list of genetic disorders amenable to treatment via this approach is regularly expanding. Research trials are in active progress for synthesis of recombinant enzymes for other genetic errors due to enzyme deficiencies like storage diseases and many others.

In mild conditions, **provision of enzyme co-factors or activators**, mostly vitamins, in pharmacologic doses, might result in restoration of effective enzyme activity with remarkable improvement. This approach underlies the well established use of **thiamine** in maple syrup urine disease, of **pyridoxine** and **vitamin C** in homocystinuria, of **vitamin B12** in methylmalonic aciduria, and many other metabolic errors.

The finding that post-translational modifications defects of many enzymes and proteins are attributable to deficient or defective functions of restorative **chaperones, a subfamily of heat shock proteins**, secondary to mutations affecting one or more of the chaperon genes family, have opened a wide and promising therapeutic prospective **(Bukau et al, 2006)**. This approach aims at either provision of these chaperones or correction of the genes producing them as a new modality for combating this pathogenetic mechanism and treatment of diseases caused by this pathophysiological alteration.

Pharmacological chaperones are small molecules that specifically bind to and stabilize the functional form or three-dimensional shape of a misfolded protein in the endoplasmic reticulum (ER) of a cell. When misfolded because of a genetic mutation, the protein (or enzyme) is unable to adopt the correct functional shape. This misfolded protein is recognized by the quality control system in the cell, and destroyed, leading to decreased amounts of enzyme that gets transported from the cell's ER to the cell's lysosome, hence, reduced enzyme activity. The binding of the chaperone molecule helps the protein fold into its correct three-dimensional shape. This allows the protein to be properly trafficked from the ER and distributed to the lysosome in the cell, thereby increasing enzyme activity and cellular function, reducing substrate and stress on cells.

**Organ, Tissue, Cell replacement and transplantation**

**Replacement (transplantation) therapy** is a wide term that encompasses too many varied applications. The concept of replacement therapy is a logic one based on the known pathogenetic mechanisms of most genetic disorders. Insufficient production of a gene product or its production in a defective way can be corrected by replacing the deficient or the defective product with a normal natural or synthesized one. This reasoning demarcated the narrow-scale use of the concept in hormone replacement therapy for endocrinal disorders, the use of immunoglobulin preparations for humoral immunodeficiency states, the use of anti-hemophilic globulin for hemophilia, the use of recombinant human enzyme preparations for many enzyme-deficient conditions, and similar allied disorders.

Extension of the concept of replacement therapy to a wide-scale applications resulted in a revolutionary turn in medical practice. Thus, a whole organ might be replaced as in **organ transplantation**. In **tissue transplantation**, a part of an organ is replaced with a healthy one like cardiac valve transplantation, cornea transplantation, and bone grafting. **Cell infusion** or replacement, like whole or fractionated blood transfusion or **stem cell therapy** is, in a sense, a transplantation technique. However, in stem cell transplantation the goal is to offer affected patients with a life-long chance for these cells to replace defective cells, damaged tissues, or failing organs.

Despite remarkable achievements in this field, chronic allograft rejection, the side effects of the long-term immunosuppressive treatment and organ shortage are still the major obstacles to achieving long-term survival. Precautionary measures needed to ensure success of replacement therapy stem mostly from fears of rejection of transplanted, infused or injected foreign material. However, advances in immunogenetics allowed for better prognoses by refining selection of compatible donors, and expected advances in genetic manipulation techniques aiming at prediction or detection of rejection at its earliest stages (**D. Sankaran et al., 1999**), nullifying foreign antigen processing and detection, and induction antigen-specific tolerance would surely lessen the risk of rejection and related complications to a minimum (**Gudmundsdottir and Turka, 1999**) and (**Jessamyn et al., 2002**).

One major complication facing organ transplant recipients is the requirement for life-long systemic immunosuppression to prevent rejection. the advent of immuno-suppressive drugs participated actively in ensuring the success of transplantation therapy, however, their use implies potential major side effects to exposed patients which are associated with an increased incidence of malignancy and susceptibility to opportunistic infections. Many gene therapy techniques have been advented to reduce and obviate these risks. Gene therapy has the potential to eliminate problems associated with immunosuppression by allowing the production of immunomodulatory proteins in the donor grafts resulting in local rather than systemic immunosuppression. Alternatively, gene therapy approaches could eliminate the requirement for general immunosuppression by allowing the induction of donor-specific tolerance. Gene therapy interventions may also be able to prevent graft

damage owing to non-immune-mediated graft loss or injury and prevent chronic rejection (**J. Bagley and J. Iacomin, 2003**).

Another major challenge to transplantation approaches is failure of transplanted organ, tissue, or cells to maintain their own survival. Enhanced apoptosis of transplanted organs or tissues is a common complication of transplantation therapy. Furthermore, some of the immunosuppressive drugs currently in clinical use might exert their activity at least in part through effects on apoptotic pathways. From the available data, it can be inferred that apoptosis contributes to the outcome after organ transplantation, being involved both in graft rejection and in transplantation tolerance (**Kabelitz Dieter, 1998**). Many genetic therapies were tried to lessen and obviate this risk with promising success, for instance, the use of heme oxygenase-1 gene transfer to prolong survival of cardiac allograft (**C Braudeau et al, 2004)** and use of anti-apoptotic genes for prolonging survival of pancreatic islets transplantation **(Contreras et al, 2001).**

## Pharmacological Therapy

Like most other diseases, **pharmacological or drug therapy of genetic diseases** occupies a pivotal role in current, and expected, management of most of these diseases. The spectrum of this therapeutic approach is very wide and encompasses a very long list of both **pharmaceutical chemicals and pharmaceutical biological preparations**. Chemical drugs used widely in management of genetic disorders include **anti-epileptics** for genetically-determined seizures, **lipid lowering drugs** for genetic hyperlipidemias, **Nitisinone** (**NTBC**) for treatment of Tyrosinemia type I, **hydroxyurea** and **piracetam** for treatment of sickle cell anemia, **trimethyl glycine** (**Betaine**) for treatment of homocystinuria, the iron chelator **deferoxamine mesylate** for treatment of thalassemias and similar chronic hemolytic conditions, and too many other genetic disorders.

Similarly, pharmaceutical biological preparations have a major role in treatment, alleviation and prophylaxis of genetic diseases and their complications. Examples of these preparations include: use of **immunoglobulins** in treatment of humoral immunodeficiency states, use of **megavitamins** therapy in management of many metabolic errors like B1 in maple syrup urine disease, B6 in homocystinuria, Folic acid for prevention of neural tube defects and use of α-tocopherol in Ataxia with vitamin E deficiency (AVED) (**Schuelke M et al, 1999**). Additional examples include the use of other synthetic **vitamins**, **hormones**, **enzymes** and **proteins** for treatment of genetic diseases due to defective or deficient production of these biological active and essential components.

## Surgical intervention

**Surgical intervention** for treatment of birth defects and possible developing defects in genetic disorders plays a very important role in management of these diseases. Without such an approach, the life of patients with surgically-correctable congenital malformations would have been a real misery. The same guidelines applied for these procedures in non-genetic diseases apply also for genetic diseases. The list of congenital defects amenable for surgical correction either for radical cure or for alleviation of sufferings and prophylaxis against worse downhill progression is too long, examples of candidate conditions include congenital heart diseases, congenital urinary tract obstructions, congenital neural tube defects, congenital bone dysplasia and malformations, congenital defects of external genital organs, and many congenital ocular and auditory malformations.

**Fine surgical intervention**, also, is practiced for many genetic defects with considerable success. For instance, treatment of genetically-determined intractable seizure foci by bipolar electro-coagulation of functional cortex (**Guoming Luan et al, 2001),** use of isolated or combined fine **laser treatment** for ocular visual defects and dysplastic conditions of the cornea, the retina and the choroids, implantation of smart microchips for peripheral vaso-occlusive and myocardial diseases, and the use of **robotic surgery** for more safe and effective surgical management of many malformation disorders reveal the role of fine or micro-surgical procedures in this therapeutic approach.

## Fetal therapy

**Fetal therapy**, or treatment of genetic diseases or malformations of the fetus during intra-uterine life, represents a crucial approach to avoid progression of such diseases or malformations to a hopeless situation if left without intervention till birth. **Surgical intra-uterine management** of some fetal conditions either via open fetal surgery or minimally-invasive fetoscopic surgery has an important role in treatment of many fetal malformations. Intervention in hydrocephaly, many types of spina bifida, congenital diaphragmatic hernia, urinary tract obstruction and some congenital heart defect illustrates few applications of this approach in fetal therapy.

**Fetal drug therapy** and prophylaxis also has a crucial role in treatment and / or prevention of a large number of genetic fetal disorders. Examples of such applications include use of corticosteroids prevention of external genital masculinization in female fetuses with 21-hydroxylase deficiency syndrome (**Cerame BI et al., 1999**), biochemical amelioration of methylmalonic acidemia and biotin-responsive multiple carboxylase deficiency, control of fetal cardiac arrhythmias with Amiodarone (**Janette Strasburger et al., 2004**), and treatment of fetal fetal goitrous hypothyroidism with L-Thyroxine (**H. Hashimoto et al., 2006**).

## Gene therapy

As the term implies, gene therapy aims at offering radical treatment of genetic diseases via correcting the underlying pathogenetic mechanisms of these diseases at the gene level. Based on our knowledge of these mechanisms, a wide variety of techniques have been theorized. Unfortunately, quite few of them are worthy of trial. With the exception of the success of classic viral-based gene therapy for **malignant melanoma** of the skin (**Maria Sotomayor et al, 2002)** and the success of stem cell therapy for some selected genetic disorders, the way to safe and successful gene therapy is still very long.

Current trials of gene therapy techniques comprise many different approaches. Some of them target the deficient or defective gene function by trying to compensate for the lost function by offering normal counterparts of the diseased gene to affected cells or tissues through e.g. **gene transfer mechanisms.** Other techniques target the whole affected cells by offering normal cells with normal whole genomes, like the case in **stem cell therapy**. In between these two extremes of the spectrum, too many other techniques are being tested with hopeful expectations. These approaches aim at targeting underlying pathogenetic mechanisms at all possible stages, for example by manipulation of post-transcriptional effector molecules like mRNA by micro- or small- RNAs, manipulation of the translation machine in the cytosol by signal transducers or by manipulating post-translational events by chaperones. Such techniques include, for instance, the use of the ability of **hammerhead ribozyme** to induce site-specific cleavage of RNA to down-regulate the expression of mutant alleles (**LA Phylactou et al., 1998), the** use of **RNA interference** technology (iRNA) for control of underlying defects in movement disorders (**Hideki Mochizuki et al., 2008),** the use of nanoparticles, instead of viral vectors, as gene carriers to target cells for many diseases, e.g. lung cancer where nanoparticles are used to target cancer cells with dual tumor suppressor genes (**Jack Roth and Lin Ji, 2007),** the induction of **molecular chimerism** as a potent regulator of cell functions to alter cell behaviour e.g. induction of B-cell and T-cell tolerance for preventing graft rejection and prolonging survival of transplanted organs **(Iacomini and Bracy, 2001 & Bracy JL et al., 2001)** and use of **pharmaceutical molecular chaperones** to correct post-translational modifications defects which underlie the pathogenesis of a considerable number of common and serious genetic diseases like Alzheimer's disease, Parkinson's disease, Huntington's disease, Creutzfeldt-Jakob disease, cystic fibrosis, Gaucher's disease and many other degenerative and neurodegenerative disorders (**Tapan and Subhankar, 2006**).

## Therapeutic approaches to genetic diseases

### General guidelines

**1.** In the vast majority of genetically-determined disease states, **combined therapeutic approaches** are needed often to accomplish the best therapeutic and prognostic results. According to the nature of each disorder, such combined approaches might synergize to **counteract underlying pathogenetic mechanisms**, to **limit progressive damage** caused by continuing exposure to genetic and/or environmental insults or by continued accumulation of toxic substrates or metabolic intermediate products, to **combat resulting metabolic or pathophysiological alterations**, to **supply deficient gene products** or other biomolecules, or to offer near radical cure in some conditions, e.g. via organ transplantation. So, **proper evaluation and selection of all necessary treatment strategies** of a specific disease, in order not to ignore any of them, is of utmost importance as a first step in determining the management plan of the condition in question.

**2.** The underlying pathogenetic mechanism(s) and the resulting pathophysiological alteration(s) of each disease determine the **proper therapeutic approaches** as well as the **priority of each intervention modality**. For example, restriction of protein or carbohydrate intake constitutes the mainstay of therapy of errors due to defective metabolism of amino acids or specific sugars respectively, with removal of the resulting toxic metabolites occupying next priority in management. The reverse seems more practical for errors like chronic hemolytic anemia or hemochromatosis where removal of the excess iron overload through chelation therapy, rather than restriction of dietary consumption of iron-rich foods, is the principal goal of treatment.

**3.** Also, the availability and overlap of many different treatment approaches to most genetic disorders necessitates a **co-operative team approach** of different specialties in order to achieve the best therapeutic and/or prophylactic prospects. In addition to the **medical geneticist**, and depending on the nature of each disease, the team might include a **biochemical geneticist**, a **specialized laboratory doctor**, a **dietitian** specialized in dietetic management of metabolic disorders, a **pharmacist** familiar with drug/formula preparations commonly used in genetic diseases, and a **plastic surgeon** experienced in surgical management of congenital malformations. The contribution of surgical intervention depends on the situation met with, so a cardiac surgeon is resorted to for congenital heart diseases, a neurosurgeon is referred to for CNS anomalies, a urosurgeon consultation is asked for genital anomalies, and so on. Conditions necessitating organ transplantation, for instance, will need a much more diverse team work approach to guarantee the best results aimed at. This **concept of team work approach** in management of genetic disorders is crucial for success of therapy since it offers the best conditions of medical and / or surgical care that can be availed to the patient.

**4.** Although the aforementioned therapeutic approaches constitute the mainstay of management of genetic disorders, the role played by **supportive intervention measures** can not, and should not, be ignored or overcome. Such supportive measures include **physiotherapy**, **speech therapy**, **prostheses**, **hearing aids**, **visual aids** and **orthopedic devices** which are used for treatment and or prophylaxis of skeletal deformities like scoliosis, camptomelia, talipes and many others. The same also applies for trends aiming at institution of **early intervention protocols** and **rehabilitation programs** for patients born with genetically-determined mental and / or physical handicaps, or patients who have lost the ability to function normally, often because of a progressive genetic disorder like neurodegenerative disorder, muscle dystrophy or skeletal dysplasia.

## Review of literature

## 1. Substrate restriction/removal approach

Dietary restriction is one of the most effective methods of managing genetic diseases that result from inborn errors of metabolism of dietary constituents. This therapeutic approach usually requires lifelong compliance with restricted, and often artificial, diet (**O'connor and Crystal, 2006**). Many of the diseases treated in this manner involve amino acid catabolic pathways, and therefore strict restriction of normal dietary protein is usually necessary. Essential nutrients such as amino acids, however, cannot be withheld entirely; their intake must be sufficient for anabolic needs. For that group of patients who have mild enzymatic defects small amounts of the offending compound can often be tolerated; consequently, the diet is less restrictive, and compliance may be better (**Treacy et al, 2001**). Table (1) shows some examples for the use of dietary restriction as a therapeutic modality in treatment of this category of genetic diseases.

**Table (1): Examples of dietary restriction approach in genetic disorders (Peter and Sian, 2007)**

| Disorder | Example |
|---|---|
| Disorders of amino acids metabolism | A-Phenylketonuria<br>B- Urea cycle defects<br>C- Maple syrup urine disease<br>C- Tyrosenimia type I |
| Disorders of carbohydrate metabolism | I. Diabetes mellitus<br>II. Galactosaemia |
| Disorders of organic acids metabolism | A-Glutaric academia type I<br>B-Methylmalonic academia<br>C-Isovaleric academia |
| Dietary restriction in hyperlipidemias | A- Familial hypercholesterolemia<br>B- Familial hypertriglyceridemia<br>C- Familial combined hyperlipidemia<br>D- Familial hyperchylomicronemia |
| Dietary restriction in mineral disorders | I- Iron restriction in Hemocromatosis & Thalasemia<br>II- Copper restriction in Wilson disease |
| Disorders of purine metabolism | Dietary control in Gout |
| Dietary restriction in Celiac disease | |

Special formulae are now available for many genetic diseases including Phenylketonuria (PKU), Maple syrup urine disease (MSUD) and Tyrosenimia type I. Clinicians must be aware that the special formulae are incomplete and one should seek a dietitian experienced in the care of infants and children with these genetic diseases to ensure adequate intake of essential amino acids, fatty acids, minerals and vitamins.

Indiscriminate use of special formulae without appropriate supplementation or strict protein restriction can result in an acrodermatits entropathica-like syndrome, which may be secondary to deficiency of trace metal such as zinc, essential amino acids, and essential fatty acids (**Giacoia and Berry, 1993**). Regular monitoring of plasma metabolite concentrations would be informative regarding dietary compliance, and is essential for dietary adjustment.

**Management of Phenylketonuria (PKU)**

Phenylketonuria (PKU) is an autosomal recessive inborn error of phenylalanine (Phe) metabolism resulting from deficiency of phenylalanine hydroxylase (PAH) enzyme that converts phenylalanine to tyrosine (Fig. 1). PKU is caused by many genotypes that cause a spectrum of affection ranging from classic severe type to milder types, e.g. tetrahydrobiopterin-responsive PKU. Untreated PKU is associated with growth failure, poor skin pigmentation, microcephaly, seizures, global developmental delay, severe intellectual impairment and many other abnormalities. However, since the introduction of newborn screening programs and with early dietary intervention, children born with PKU can now expect to lead relatively normal lives (**Hanly, 2004**).

Management of phenylalanine rests on the same basic principles followed in management of similar metabolic errors due to defective metabolism of ingested food substrates; viz restriction of intake of offending stuff, removal or neutralization of toxic by-products and supplementation of deficient vital metabolites.

**Potential Nutritional Deficiencies in PKU**

The Phe-restricted diet with semi-synthetic supplementation is not without risk. PKU patients under dietary treatment can have low concentrations of trace elements and cholesterol and some disturbance of folate metabolism as well as distortion of their fatty acid profile (**Lucock et al., 2002**) (**Schulpis et al., 2004**). However,

it remains controversial as to whether all of these dietary imbalances occur as a result of insufficient intake and supplementation, or are due to endogenous disturbances in the biosynthesis of these essential constituents.

**1- Dietary restriction of phenylalanine**

Normal blood phenylalanine levels are 58 +/- 15 µmol/L in adults, 60 +/- 13 µmol/L in teenagers, and 62 +/- 18 µmol/L in childhood. In the newborn, the upper limit of normal is 120 µmol/L (2 mg/dl). In untreated classical PKU, blood levels as high as **2.4 mM/liter** can be found (**Scriver et al, 1985 & Gregory et al, 1986**).

The mainstay in treatment of PKU is a low-Phe diet which, by reducing or normalizing Phe concentrations, prevents the development of the neurological and psychological changes in affected patients. Since neurological changes can be demonstrated within one month of birth, it is recommended that dietary restriction should be started early and be continued through childhood when neural development is maximal (**Konechi et al, 1992**). Clinical neurological abnormalities, affected neuropsychological performance and brain imaging in adults with PKU has led to a consensus opinion that the PKU diet should be followed for life (**The National Society for Phenylketonuria, 2004**).

A low Phe. diet is used for treatment with, initially, the small amounts of Phe. coming from breast milk or commercial infant formula considered sufficient intake in babies. In older children, protein intake is calculated each day, whereby a child is allocated a certain number of grams or units of daily protein, depending upon plasma Phe concentrations. Foods such as eggs, milk, cheese, meat, poultry, fish, dried beans and legumes which are high in protein are excluded from the diet (**Michals, 2001**). However, this regime would not normally provide enough protein for growth requirements and therefore, commercially available supplements of essential amino acids, lacking Phe, need to be taken on a daily basis; for example Lofenlac formula. Regular blood Phe assays which, in concert with complete food diaries, are used by dietitians to make necessary adjustments to the recommended diets (**Michals, 2001**).

The main benefit of the Phe-restricted, low protein diet, is avoidance of the neurotoxicity caused by hyperphenylalaninemia with consequent prevention of neurological damage and improvement of neurological and psychological performance. However, dietary treatment does not come without challenges such as compliance with the diet, the requirement of social support, and risk of imbalances in essential dietary nutrients (**Hanley, 2004**).

The best indicator of dietary compliance in classical PKU is normalization of blood Phe concentrations. Phenylalanine levels are followed at regular intervals, from 1-2 times weekly in neonates to once per month in older children and adults. Most US facilities recommend that phenylalanine levels be maintained at 2-6 mg/dL (120-360 µmol/L) (**Moseley et al, 2002**).

**Emerging PKU therapies**

**1- Tetrahydrobiopterin ($BH_4$) Therapy**

Recent clinical trials have shown that a subset of 'classic' PKU children respond to $BH_4$ therapy, dependent upon their PAH gene mutation(s) (**Hennermann et al, 2005**). Sapropterin dihydrochloride (Kuvan, Biomarin Pharma) is an orally active synthetic form of $BH_4$ used in treatment of $BH_4$-responsive PKU. Previous results of using Kuvan have shown that it is a safe and effective therapy in selected patients with HPA and mild-to-moderate PKU who responded to a $BH_4$ loading test (**Burnett, 2007**).

**2- Neutral Amino Acid (NAA) Therapy**

Competition for the Phe l-type amino acid carrier by other NAAs may occur in PKU. This hypothesis has led to NAA supplementation therapeutic trials in PKU where increasing the blood concentrations of various NAAs has led to reduced brain concentrations of Phe (**Rietz et al, 1999**). Furthermore, the increased tyrosine (Tyr) and tryptophan (Trp) intake may be of benefit in disorders of $BH_4$ regeneration. A new NAA formula (NeoPhe, Solace Nutrition) has been found to be effective in reducing blood Phe concentrations (**Matalon et al, 2006**).

**3- LCP (Long-chain polyunsaturated fatty acids)**

Recent research indicates that providing supplements of specific fatty acids, namely docosahexaenoic acid (**DHA**) and arachidonic acid (**AA**), to infants, pregnant women and individuals with certain metabolic disorders may offer preventive and therapeutic benefits, especially in relation to brain development, retinal integrity and visual-cognitive functions. In addition, supplementing both fatty acids together in prescribed amounts may augment their effects on neurotransmission and membrane maturation. Recently, a small group of experts has concluded that infant formulas for term infants should contain at least 0.2% of total fatty acids as DHA and 0.35% as AA, while formulas for preterm infants should include at least 0.35% DHA and 0.4% AA. All recent reviews and recommendations have underlined the lack of adverse effects from these supplementations. In addition, providing supplements of DHA to pregnant women was recently shown to be associated with improved early developmental outcome of the offspring.

Infants and children with phenylketonuria may benefit from diets supplemented with long-chain fatty acids, especially DHA, AA and eicosapentaenoic acid (EPA.) Providing these supplements to pregnant women and to patients who are not in good compliance with a PKU diet may also have some benefit (**Galli et al, 1991**).

In fact, well-treated hyperphenylalaninemics have low levels of LCP (long-chain polyunsaturated fatty acid), particularly DHA, in plasma and red blood cells. The addition of LCP to the diet of older children with phenylketonuria, through supplementation with fish oil for three months or with specific preparations containing AA, EPA and DHA for one year (13), raises plasma levels of these fatty acids and improves visual response.

Blood AA levels, along with blood phenylalanine appear to be predictors of the neural performance of children with HPA at 12 months of age. Along with HPA infants and children, pregnant women with HPA and HPA adults who are not compliant with their diet treatments might benefit from LCP supplementation. Providing LCP supplements to pregnant women should help assure maximal placental transfer of LCPs during the last three months of pregnancy. The provision of DHA supplements to poorly compliant patients should increase levels of neuroprotection and lessen the theoretical risk of inhibiting their own LCP synthesis with toxic phenylalanine byproducts (**Galli et al, 1991**) and (**Koletzko et al, 2007**).

**4- Phenylalanine ammonia lyase (PAL) trial therapy**

PAL acts as a substitute for the enzyme phenylalanine mono-oxygenase which is deficient in PKU. PAL, a robust enzyme without need for a cofactor, converts phenylalanine to metabolically insignificant amounts of ammonia and trans-cinnamic acid, a harmless metabolite. the latter is converted to benzoic acid and rapidly excreted in urine as hippurate. Animal studies and studies in human PKU patients showed analogous responses after the administration of PAL in enteric-coated gelatin capsules, and revealed promising results for this different therapeutic aspproach to PKU, the main drawback is its unaccepted high cost. However, a recombinant PAL has been synthesized and continuing researches point to a promising role expected to be exerted by this new approach in management of PKU (**Christineh Sarkissian et al., 1999) and Sarkissian and Gámez, 2005).**

**Management of Maternal PKU**

Highly elevated concentrations of Phe are teratogenic and are a cause of increased risk of miscarriage (American Academy of Pediatrics, 2001). Specifically, the fetus can be affected by elevated Phe concentrations which lead to intrauterine growth retardation, facial dysmorphism, microcephaly, congenital heart disease and developmental delay. Maternal hyperphenylalaninemia can underly the existence in involved families of multiple cases affected with birth defects and intellectual impairment (Knerr et al, 2005) (Show- Smith et al, 2004).

Because of the fetal morbidities associated with hyperphenylalanine, a more stringent control of blood Phe concentrations is required in mothers with PKU. A preconception diet is required with a Phe target interval of between 100 and 360 μmol/L in affected mothers (Lee et al, 1978). In addition, weekly monitoring of the Phe concentrations is advised to aid in achieving low baseline levels (Dennison, 2005).

**Management of Urea cycle defects**

The urea cycle disorders (UCD) are a group of diseases that result from various disease-specific enzymatic defects leading to disturbed metabolism of the extra nitrogen produced by the breakdown of protein and other nitrogen containing molecules. They are among the most common inborn errors of metabolism with a cumulative incidence approximating 1 in 8000. UCD can present at all ages, are easy to diagnose but frequently overlooked and, in principle, treatable diseases. The emergency assay of blood ammonia must be a part of the basic emergency investigations in all patients with undiagnosed encephalopathy at any age.

UCD are characterized by the accumulation of ammonia and other toxic precursor metabolites upon breast milk or protein intake during the first few days of life. Infants with a urea cycle disorder often initially appear normal but rapidly develop cerebral edema and related signs of lethargy; anorexia, hyperventilation or hypoventilation, hypothermia, seizeures, neurologic posturing and coma (**Bourrier et al., 1988**).

**A. Dietary management**

**1. Protein restriction**

Long-term management is focused on restriction of dietary protein through use of specialized formulas and strict maintenance of a positive anabolic state. This is attained by limiting protein intake to minimal requirements as normal protein. If necessary, natural protein containing approximately 50 % essential amino acids can be replaced by half the amount of an essential amino acids mixture.

**2. Dietary supplementation**

- In ornithine transcarbamylase deficiency and in carbamyl phosphate synthetase deficiency, **Arginine** is supplied in 100-200 mg/kg/d. Citrulline in the same dose is a more potent alternative in severe cases of both disordes.
- In Citrullinemia due to argininosuccinate synthetase deficiency and in Argininosuccinic aciduria due to argininosuccinate lyase deficiency, **Arginine** is supplied in a dose of 600 mg /kg/d.
- **Vitamins**, including folic acid 500 μg/d, **trace elements** and **Carnitine** 30-50 mg/kg/d if low.

**B. Pharmacological therapy**

Drug therapy is crucial in UCD to remove accumulated ammonia. It depends on use of nitrogen scavenging drugs: oral Sodium benzoate or sodium phenylbutyrate in doses of 250-500 mg/kg/d, and Lactulose that binde intestinal ammonia in dosese up to 4-20 grams three times per day (**Zschocke and Hoffmann, 1999)**. Home care is recommended with gastrostomy tube feedings as necessary, and minimizing risk of intercurrent respiratory and gastrointestinal illness by antibiotics is mandatory (**Summar, 2001**).

**C. Hemodialysis-Peritoneal dialysis**

Hyperammonemia is one of the most urgent emergencies in metabolic medicine and necessitates immediate and aggressive management. Dialysis represents the most efficient acute management approach for acute hyperammonemia. Following stabilization of the case, the above mentioned therapeutic approaches are immediately instituted.

**Management of maple syrup urine disease (MSUD)**

Maple syrup urine disease (MSUD) is an autosomal recessive amino acidopathy secondary to enzymatic defect(s) in catabolism of the branched chain amino acids **(BCAAs)**: leucine, isoleucine, and valine. Accumulation of these three aminoacids and their corresponding ketoacids leads to encephalopthay and progressive neurodegeneration in untreated infants. Early diagnosis and dietary intervention prevent complications and may allow for normal intellectual developmental. Consequently, MSUD has been added to many newborn screening programs, and preliminary results indicate that asymptomatic newborns with MSUD have better outcome compared with infants who are diagnosed after they become symptomatic.

The mainstay in the treatment of MSUD is long-term dietary restriction of branched chain amino acids as well as treatment of the frequent episodes of acute metabolic decompensation that characterize this disease following stresses like infection, dehydration, or acidosis (**Harris et al., 2005**).

**1. Dietary therapy**

The goal of dietary therapy is normalization of blood levels of branched-chain aminoacids particularly leucine by restricting the intake of branched chain amino acids without impairing growth and intellectual development. Dietary therapy must continue for life.

Typically, the MSUD diet does not include any high protein foods. The major component of the diet is a special formula designed for infants, children or older adults with MSUD. These formulas do not contain any leucine, isoleucine or valine but are otherwise nutritionally complete. They contain all the necessary vitamins, minerals, calories and the other essential amino acids needed for normal growth and development (**Morton et al., 2003**).

However, people with MSUD require some leucine, isoleucine and valine (as they are essential amino acids) and thus the diet is supplemented with special low protein foods and weighed or measured amounts of vegetables, fruits and some grains. These types of foods provide the necessary amount of branched chain amino acids based on the patient's specific presentation of the disease. These foods must be measured or weighed as the quantity of **BCAAs** allowed in the patient's diet are based on their individual tolerance for leucine, isoleucine and valine. Some children can have fairly liberal diets and still maintain good control of blood **BCAAs** levels, while others must follow a very strict diet.

Several commercially available foods and formulas without, or with, reduced levels of **BCAAs** are available for juveniles and adults, such as MSUD express, Enfamil and Similac. The intake of leucine is calculated on an individual basis following the measurement of plasma branched chain amino acids on regular basis at appropriate intervals for life.

**2. Thiamine supplementation**

Many cases of MSUD are due to enzyme misconfiguration defects that can be reversed with therapeutic doses of Thiamine that apparently acts as an essential cofactor for the enzyme. For any person with MSUD in whom the functional consequences of the mutation (s) are unknown, a four-week trial of enteral thiamine (50-100 mg/day, divided twice a day) is reasonable. However, it should be noted that significant changes in dietary therapy (e.g., BCAA or calorie intake) during the treatment period confounds interpretation of a specific thiamine effect (**Morton et al, 2003**).

**Management of Tyrosinemia type I**

Tyrosinemia type I is a autosomal recessive disorder that results from a mutation in the gene fumaryl-acetoacetase (FAH) gene responsible for synthesis fumarylacetoacetase needed to metabolize (or breakdown) the amino acid tyrosine. Mutations of the FAH gene result in deficient/defective production of fumarylacetoacetase, which results in a failure to breakdown tyrosine. Abnormal accumulation of tyrosine and its metabolites, viz : fumarylacetoacetate, succinylacetoacetat, maleylacetoacetate and succinylacetone, occurs in various organs with resulting deleterious effects that characterize the disease (**Berger et al, 1981**).

Succinylacetone has been demonstrated as a mitochondrial toxin that inhibits substrate-level phosphorylation by means of the kerbs cycle. It is, also, a potent inhibitor of gamma-aminolevulinic acid dehydratase, the enzyme that

mediate the formation of porphobilinogen, the cyclic precursor of prophyrins in the heme biosynthetic sequence. The accumulated metabolites including fumarylacetoacetate, succinylacetoacetate and succinylacetone are potent alkylators of DNA, which account for their ability to induce mitotic abnormalities in the genome and their carcinogenic potential (**Jorquera and Tanguay, 2001**).

**1. Dietary therapy**

Most patients are so ill at the time of presentation that inpatient treatment is mandatory. Nutritional treatment should be designed to minimize the phenylalanine-tyrosine load to only essential requirements (**Ashorn et al, 2006**). All children should be prescribed a low phenylalanine low tyrosine diet designed to meet their needs for growth without providing excess of these amino acids.

**2. Drug therapy**

The use of Nitisinone (NTBC) (2-nitro-4-trifluoromethyl-benzoyl-1,3-cyclohexanedione, a highly potent inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase, in a dose of 1-2 mg/Kg/d in 2 divided doses, has been approved as a complementary step in management of tyrosinemia type I. NTCB prevent the formation of fumarylacetoacetate from tyrosine and blocks the accumulation of the toxic metabolites thus reducing their toxic effects on body organs and tissues (**Koelink et al., 2006**).

**3. Liver transplantation**

Due to the carcinogenic potential of the toxic metabolites, infants affected with tyrosinemia type I are at a high riask of developing hepatocellular carcinoma, oftenly before 2 years of age. In severe refractory cases not responding to dietary and pharmacokogical treatment, liver transplantation is a life-saving approach if performed early enough in the course of the disease. It normalizes the metabolic defects, prevents the development of renal failure and obviates the dreadful consequences of carcinogenesis and hepatic malignancy (**Büyükpamükukcü et al., 2006**).

**Management of galactosemia**

Hereditary galactosemia is among the most common carbohydrate metabolism disorders and can be life threatening illness during the newborn period (**Segal, 1995 and Berry et al., 2006**)

The mainstay of medical care in the postnatal period is to immediately discontinue ingestion of lactose-containing formula. This ameliorates the acute toxicity associated with the neonatal period but does not prevent all long-term complications. Clotting abnormalities may be cryptic and require fresh frozen plasma treatments (**Levy et al., 1996**).

Galactose restricted diet should be prescribed for galactosemic infants. Older patients may tolerate galactose better than infants. The restriction of milk intake throughout life is controversial. However, most metabolic specialists support a life-long diet therapy (**Walter et al, 1999**). Total elimination of galactose is difficult because it is present in a wide variety of foods (eg, infants foods, fruits, vegetables), especially in the macromolecular form and many lactose-free foods are known to contain free galactose (**Garden and Davidson, 2000**). Because alternative soy formulas are poor in calcium sources, adequate calcium intake throughout the dietary management of galactosemic patients should be supplied.

**Therapeutic Approaches To organic acidurias (OA)**

The organic acidurias comprise a diverse group of autosomal recessive disorders, with some of them resulting from mitochondrial defects, characterized by excretion of organic acids in urine. The most important OA result from dysfunction of specific enzymatic steps in catabolism of branched-chain amino acids. Their characteristic pathophysiological alterations results from the accumulation of the harmful precursors and deficiency of needed products of the affected pathway(s). Accumulation of CoA metabolites in the mitochondria is an important finding which differentiates between many OA and aminoacidopathies. Because catabolism of amino acids provides energy for other cellular processes, energy deficiency during metabolic crises may contribute to the clinical syndrome (**Hoffman and Zschocke, 1999**).

**Guidelines of management of OA**

- In acute exacerbations: **stop protein intake**, **reduce accumulation of toxic metabolites** by intestinal antibiotics (metronidazole 10 mg/kg or Colistine), **block catabolic state** with high-dose energy supply : 10 % glucose infusion at a rate of 150 ml/kg/d (~10 mg/kg/min,~60 kcal/kg/d) and parenteral lipids at a dose of 1 gm/kg/d, **adequate fluid and electrolyte intake** and maintenance of sodium concentration at ≥ 140 mmol/L to obviate the risk of cerebral edema, enhance diuresis and if needed, dialysis and hemofiltration might be resorted to.
- Long-term management of classical OA includes the use of specific formulas deficient in the particular precursor amino acid for each disorder, as it provides the essential amino acids in otherwise a protein-restricted diet (0.5-1.5 g/kg/d), adequate calories to inhibit catabolism supplied as carbohydrates and fats, addition of L-Carnitine (50-100 mg/kg/d), L-Glycine (150-250 mg/kg/d) for isovaleric aciduria, B12 (25 µg/kg/d) for methylmalonic aciduria and Metronidazole 10 mg/kg/d or Colistine for 10 days every month to reduce enteral

propionic acid accumulation and absorption. Total parenteral nutrition has been used during gastrointestinal illness or surgery but must be monitored with careful attention to biochemical parameters (**De Baulny et al, 2005**).

- Strict adherence to management protocols is crucial for cerebral OA (glutaric aciduria type I, Canavan disease, Ethylmalonic encephalopathy, and other less frequent types) in addition to use of antiepileptic medications and symptomatic supportive measures (**Kolker et al., 2006**).

**In Glutaric aciduria type I,** dietary restriction comprises low protein diet restricted in lysine/tryptophan. hyroxytryptophan, the precursor of serotonin that is not metabolized to glutaryl-coA, glutaric acid and secondary metabolites, could be used as an adjuvant to selective tryptophan restriction However the evidence in favors of selective tryptophan restriction remain insufficient and the consequences evolve towards the restriction of lysine only (**Christensen et al., 2004**). Also a better outcome was obtained with a low-protein diet supplemented with lysine-free special formulas enriched in micronutrients as compared to protein restriction alone (**Kolker et al., 2004**). Supplementation of carnitine (100 mg/kg/ day) are used universally and sometimes as the only treatment to keep levels of free carnitine in the high or slightly above the normal range (60-100 micromolar), to provide a buffer to aid the elimination of glutaric acids in case of acute decompensation (**Naughten et al., 2004**). Also, Riboflavin and anticonvulsant treatment are used to reduce risk of neuronal damage (http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2556991&tool=pmcentrez - R21**Muhlhausen et al., 2004**).

**Management of Methylmalonic aciduria (MMA) comprises: protein restricted diet** or a special formula restricted in precursors of methylmalonate, containing **decreased amounts of isoleucine, valine, methionine and threonine**, instituted as soon as life threatening problems such as ketoacidosis, hypoglycemia, or hyperammonemia have been addressed (**Berry, 1998**) and (**Goetz, 1999**), **B12 (Cobalamin)** I.M injections at a dose of 25 microgram/kg/day, **L-Carnitine**, 50-100 mg/kg/day and **Metronidazole** 10 mg/kg for 10 days every month to decrease enteral production of propionic acid (**Satoh et al, 1981**).

Such measures should decrease the circulating concentrations of methylmalonate and propionate. Even cobalmin-unresponsive children with delayed development have been shown to improve markedly when treated with careful dietary protein restriction (**Nyhan et al., 1973**). Supplementation with carntine is very useful to form carnitine esters with accumulated toxic CoA esters. These esters are subsequently excreted in urine (**Nyhan and Haas, 1997**).

**Management of Isovaleric Aciduria (IVA) comprises:** protein restricted diet or a special formula restricted in leucine, L-Carnitine, 50-100 mg/kg/day and L-Glycine, 150-250 mg/kg/day (**Tanaka, 1990**). The long term goal in treatment of isovaleric academia is through reduction of the production of isovaleryl-CoA from leucine catabolism through dietary manipulation (**Berry et al., 1988 & Sweetman and Williams, 2001**). Total protein and caloric intake must be adequate to support normal growth in children and maintain an anabolic state and thus monitoring of weight, length and head circumference is essential at follow up. In many cases, it may be sufficient to moderately lower protein intake with natural foods to approximately 1.5 gm/kg per day. In patients with recurrent clinical symptoms, leucine restriction in excess of total natural protein may also be necessary (**Sweetman and Williams, 2001**). Natural protein necessary to reach the recommended age-appropriate daily requirement must then be provided with leucine-free amino acids. Because of the specific role of leucine in promoting protein synthesis, however, there is a potential for adverse side effects of rigorous leucine restriction including muscle wasting (**Harris et al., 2004**).

**Management of hyperlipidemias**

**1. Familial hypercholesterolemia FH**

- Minimizes cholesterol intake: Major dietary sources of cholesterol include cheese, egg yolks, beef, pork, poultry, and shrimp. Human breast milk also contains significant quantities of cholesterol (**Jensen et al., 1978**). Cholesterol is not present in plant based food sources unless it has been added during the food's preparation. However, plant products such as flax seeds and peanuts contain healthy cholesterol-like compounds called phytosterols, which are suggested to help lower serum cholesterol levels (**Ostlund et al., 2003**).
- Replaces saturated fats (e.g. meat, palm oil, dairy products) with unsaturated fats (ex : fish, cearl, sunflower seeds). Polyunsaturated fats have some hypocholesterolemic action. When they are used in partial substitution for dietary fat they exert benefit not only by their own hypocholesterolaemic effect, but also by lowering saturated fat intake, and by improving palatability, and thus compliance. By utilizing maize or sunflower oils in cooking, and commercially available polyunsaturated margarine and cheese, an attractive diet can be devised, a major goal in managing young aged patients (**Marks et al., 2003**).
- Consumption of plant sterols and stanols can reduce plasma cholesterol (LDL-C) levels by about 10% (**Ketomaki, 2004 & Miettinen, 2004**).

- If diet regime alone is insufficient to reduce the plasma cholesterol to within normal or safe levels, or if compliance is poor, drug treatment should be advocated (**Genest et al., 2003**).

**2. Familial hypertriglyceridemia**

- Treatment includes weight reduction, dietary modification and exercise. Dietary modification should decrease weight, overall energy intake, and intakes of fat and refined carbohydrates (i.e., foods with a high glycemic index) (**Jenkins et al, 2002**).
- In severe cases associated with hyperchylomicronemia, recommended fat intake is restricted to 10-15 % of total energy intake (about 15–20 g/d), with reductions in saturated and unsaturated fats. For less severe cases of hypertriglyceridemia, restrictions to saturated and **Trans fat intake** and increased aerobic activity can reduce plasma levels of triglycerides. The National Cholesterol Education Program advises a carbohydrate intake of 55–60 % and a protein intake of 15–20 % of daily dietary intake, whereas total and saturated fat should not surpass 30 % and 7 %, respectively. Plasma triglyceride response to diet and weight loss shows marked variation among patients (**Gerhard et al., 2004**).
- Daily consumption of 4 g of omega-3 fatty acids (e.g. eicosapentaenoic and docosahexaenoic acid) which are components of both the Mediterranean diet and of fish oils along with with restricted energy and saturated-fat intakes, can reduce plasma triglyceride levels by as much as 20%. However, omega-3 fatty acids are rarely effective when used as the sole triglyceride-lowering therapy (**Hooper et al., 2006**).

**3. Familial combined hyperlipidemia**

Initial dietary management using modified fat diet for FH, together with weight reduction if indicated, will usually result in improvement but long-term dietary management is only acceptable to a minority of patients (**Hepner et al., 1979**). In these cases, drug therapy to lower lipid levels is mandatory.

**4. Familial hyperchylomicronemia**

The aim of treatment of this condition is prevention of symptoms rather than normalization of plasma lipids as familial hyperchylomicronaemia apparently does not predispose to CHD. The institution of a virtually **fat-free diet** leads to gradual clearing of the chylomicrons from the plasma over a few days, and a concomitant improvement in symptoms. Once symptoms have been abolished, some fat may be re- introduced into the diet. There appears to be a little increase in diet fat tolerance with age, and patients will probably need to remain on a quiet severe fat restriction for life. **Medium chain triglycerides** (MCT) which are found in coconut oil and palm oil are preferred as they are absorbed via the portal system and not as chylomicrons, so they can be useful diet supplements to add variety and fat energy to diet (**West et al., 1975**).

**Therapeutic approaches through mineral restriction & removal**

**1- Iron restriction in thalassemia & other chronic hemolytic anemia**

- Patients should not consume foods that contain large concentrations of bioavailable iron, such as red meats and organ meats and they should not use iron supplements, including multivitamins with iron.
- Drinking tea helps to reduce iron absorption through the intestinal tract due to its content of tannate.
- Vitamin C (ascorbic acid) increases intestinal absorption of inorganic iron. Though no reason exists to discourage patients from eating fresh fruits and vegetables containing vitamin C, they must limit ingestion of vitamin C in supplements to 500 mg/d. Although
- Vitamin C may improve iron excretion in patients receiving iron chelation. However, anecdotal reports suggest that large doses of vitamin C can cause fatal arrhythmias when administered without concomitant infusion of deferoxamine (**Peters et al., 1998**).
- The mainstay in management of thalassemia and other chronic hemolytic anemia to counteract the transfusion-induced accumulation of iron is strict removal of iron by daily administration of the parenteral iron chelator **Deferoxamine (desferal)** or the oral iron chelator **Deferasirox (EXJADE)**.

**2-Iron restriction in hemochromatosis**

- Patients should not consume foods that contain large concentrations of bioavailable iron, such as red meats and organ meats and they should not use iron supplements, including multivitamins with iron.
- Ethanol sometimes increases iron absorption, and certain alcoholic drinks, especially red wine, contain relatively high concentrations of iron. Patients with **hemochromatosis and** evidence of hepatic injury should consume no ethanol.
- Vitamin C (ascorbic acid) increases intestinal absorption of inorganic iron. Though no reason exists to discourage patients from eating fresh fruits and vegetables containing vitamin C, they must limit ingestion of vitamin C in supplements to 500 mg/d.
- Raw or improperly cooked shellfish sometimes is contaminated with V vulnificus and can cause sepsis in patients with hemochromatosis. So Seafood from potentially contaminated waters must be cooked thoroughly (Barton et al, 1998).

- Substances in foods and drinks, including tannates (in tea), phytates, oxalates, calcium, and phosphates can bind iron and inhibit its absorption.
- Removal of iron in hemochromatosis can be successfully achieved via regular **phlebotomy** which is the treatment of choice for these patients to lower their iron overload.

**3. Copper restriction in Wilson disease**

- Patients should generally avoid eating foods with a high copper content such as liver, shellfish, mushrooms, nuts, chocolate, dried fruit, dried peas, beans and lentils, legumes, avocados and bran products.
- Patients should also avoid taking copper-containing vitamin and mineral supplements.
- Drinking water from atypical sources (e.g. well water) should be analyzed for copper content and replaced with purified water if the copper content is greater than 0.2 parts per million or 100 micrograms of copper per liter (**Brewer, 2000**).
- Penicillamine, Trientine and Tetrathiomolybdate are potent copper chelating pharmacological preparations and should be used for life to prevent hazardous effects of copper accumulation.
- Zinc acetate helps prevent copper from being absorbed in the stomach and small intestine. Zinc has few side effects, but its slower copper chelating action than other medications make it an initial treatment only for pregnant women, for people without symptoms or liver damage, or for those who can't tolerate stronger medications.
- Liver transplantation may be the only option for patients with severe cirrhosis, fulminating hepatitis or other serious liver disorders.

**4. Copper restriction in vascular diseases**

Copper chelation therapy has been found to halt re-narrowing of arteries following balloon angioplasty. Preventing the function of copper in the body stops arteries from reclogging following the mechanical stress of removing arterial obstructions through angioplasty. The therapy works by limiting the cellular export of growth factors and cytokines involved in the response to injury. This new approach is a promising therapy for many arterial diseases, e.g., clogged arteries, vasculitis induced disorders and many others (**Lazar Mandinov et al, 2003**).

**Purine restriction in gout**

Gout (metabolic arthritis) is a disease created by a buildup of uric acid in blood, joints and many other organs secondary to metabolic defects in catabolism of purines via hypoxanthine and xanthine to uric acid. In this condition, crystals of monosodium urate or uric acid are deposited on the articular cartilage of joints, tendons and surrounding tissues. These crystals cause inflammation and pain, both severe. If untreated, the crystals form tophi, which can cause significant tissue damage. Gout may be primary e.g. inborn errors of purine-pyrimidine metabolism (including idiopathic), or secondary to a complication of another condition (**Vitart et al., 2008**).

Increased risk of gout was found with increased meat consumption (particulary beek, pork, or lamb) or seafood but not with consumption of purine rich vegetables or protein. A low incidence of gout was found in those with a high consumption of low dairy products (**Choi et al., 2004**).

Serum urate concentration and frequency of epsoides can be reduced by weight reduction through caloric restriction, decrease intake of carbohydrate, and increase intake of protein and unsaturated fats. Such diet also decreases plasma glucose, triglyceraides concentration and improve insulin sensitivity, thereby reducing cardiovascular morbidity and mortality. Crash dieting and fasting should be avoided (**Dessein et al., 2000**).

**Therapeutic approaches to diabetes mellitus (DM)**

DM represents a model of genetically-determined disorders where a combined therapeutic approach to attain maximal care for treatment and for prophylaxis against complications is successfully implied.

Management of DM entails **dietary restriction** of glucose-rich and lipid-rich foods, **supplementation** of vitamins and minerals, replacement of deficient insulin with either **hormonal replacement** (extracted or synthesized animal insulin or recombinant human insulin) or with glucose lowering **pharmacological drugs** and many promising trials of genetic therapy approaches like **pancreas transplantation** and **stem cell therapy**.

**Management of Celiac disease**

Celiac disease is a rare mal absorption syndrome of childhood precipitated, in genetically predisposed persons, by ingestion of gluten, the major storage protein of wheat and similar grains. Its pathogenesis involves complex interactions between gluten, immunogenetic and environmental factors so it is considered as a multifactorial disease (**Green and Jabri, 2003**).

- Nutritional therapy, the only accepted treatment for celiac disease, involves the lifelong elimination of wheat, rye, and barely from the diet. Clinical studies suggests that oats are tolerated by most of the patients with celiac disease and may improve the nutritional content of the diet and overall the quality of life (**Peraaho et al., 2004**). However oats are not uniformly recommended, because most commercially available oats are contaminated with gluten- containing grains during the growing, transportation, and milling processes (**Thompson, 2003**).

- Although wheat, rye, and barely should be avoided, there are other grains that can serve as substitute as well as other sources of starch that can provide flours for cooking and baking. Because the substitute flour is not fortified with vitamins, vitamin deficiencies may occur, they have been detected in patients who are on the diet for a lifelong (more than 10 years). Therefore, vitamin supplementation is necessary in these patients (**Hallert et al, 2006**).
- Meats, dairy products, fruits and vegetables are naturally gluten free and help to make for a more nutritious and varied diet. The elimination of gluten usually induces improvement within days or weeks, though histological recovery may be incomplete (**Lee et al., 2003**). In rare cases, children tolerate the reintroduction of normal diet after a long- term clinical and histological response (**Matysiak et al., 2007**).
- Approximately 5 percent of patients may have refractory celiac disease, defined as persistent symptoms and villous atrophy despite scrupulous adherence to a gluten-free diet. Treatment of refractory celiac disease includes nutritional support and repletion of vitamins and minerals, together with a strict gluten- free diet (**Trier, 1991**).
- The cost of gluten free products varies by the country, but the diet is usually expensive, making dietary treatment problematic for the patients with limited financial resources. There is considerable interest in the development of non-dietary therapies that may either replace or supplement the rigorous gluten-free diet. One of these trials is genetically-engineered wheat species, or wheat species that have been selectively bred to be minimally immunogenic. This, however, could interfere with the effects that gliadin has on the quality of dough.

## 2. Substrate addition/replacement approach

The significance of this therapeutic approach to genetic diseases stems from the fact that it represents the most direct and logical treatment strategy for this category of human diseases taking into consideration the fact that most of them happen because of total absence or deficient production of necessary gene products, defective synthesis of these products, or failure to undergo proper post-translational modifications. Actually, all genetic diseases without exception share this same underlying pathogenetic mechanism though via different functional and expression levels. For instance, all diseases attributed to regulatory gene disorders, also, build up secondary to deficient or defective production of regulatory micoRNAs or mediator factors. Thus, addition of deficient or defective gene products seems as the logical cure for these disorders. In fact, all available genetic treatments can be viewed within this context including, even, gene therapy trials where addition of normal functioning genes by classical gene transfer techniques, or addition of whole functioning genomes e.g. stem cell therapy or transplantation therapy, are clear, albeit indirect, examples of this substrate addition-replacement approach.
For matters of simplification and systematic delineation of treatment modalities, this approach is defined so as to include **protein** addition-replacement therapy, protein and non-protein **hormone** addition-replacement therapy and **enzyme** addition-replacement therapy. However, addition of other non-protein substrates like **vitamins**, **minerals and trace elements,** and carbohydrate/lipid **energy supplements,** as referred to frequently in the previous chapter, constitutes an important, sometimes a critical, contribution in management of many genetic disorders particularly the inborn errors of metabolism.

(**Table 2**): **Some of the most common genetic diseases that are treated/alleviated via substrate addition-replacement approach**

| Protein Replacement Therapy | Hormone Replacement Therapy | Enzyme Replacement Therapy |
|---|---|---|
| **Hemophilia A**<br>VIII-Anti-hemophilic globulin) | **Growth hormone deficiency**<br>Growth Hormone | **Gaucher disease Type 1**<br>Recombinant β-gluosidase |
| **Hemophilia B**<br>Factor IX | **Hypothyroidism**<br>Thyroxine | **Fabry disease**<br>Recombinant α-galatosidase A |
| **Multiple sclerosis**<br>Interferon β-1a & β-1b | **Insulin dependent DM**<br>(Insulin) | **Pompe disease**<br>(Recombinant α-glucosidase) |
| **Humoral immunodeficiency**<br>Immunoglobulins | **Dyserythropoietic anemia**<br>Erythropoietin | **Cystic fibrosis**<br>Recombinant inhaled DNase &<br>Pancreatic enzyme preparations |
| | **Addison disease &**<br>**Suprarenal deficiency**<br>Corticosteroids | **Phenylketonuria**<br>Phenylalanine ammonia lyase |
| | **Gonadotrophin deficiency**<br>Gonadotrophin | **α1-antytrypsin deficiency**<br>$\alpha_1$-proteinase inhibitor |
| | **Ostoeporosis** | **Enterokinase deficiency** |

| | | |
|---|---|---|
| | Parathormone | Pancreatic enzymes |
| | **Lipodystrophy & Leptin deficiency**<br>Leptin | **Adenosine deaminase deficiency**<br>Recombinant adenosine deaminase |
| | | **Hurler/Hurler-Schie syndrome Mucopolysaccharidosis type I**<br>Recombinant human α-L-iduronidase). |

**A. protein Addition/replacement therapy**

Contrary to what might seem expected, protein addition-replacement therapy is one of the most non-specific approaches in management and treatment of a wide variety of both genetic and non-genetic diseases. Protein replacement therapy might be a straightforward management when used to supply specific deficient or defective gene products, e.g. **anti-hemophilic globulin, growth hormone, lysosomal enzymes or immunoglobulins**. Non-specific use of proteins, however, constitutes the bulk of their indications in too many disease conditions comprising both genetically-determined and multifactorial-dependent disorders.

We know that although the genome defines the proteome, i.e. the genes synthesize the proteins, the proteins are the actual mediators of all life processes anf cellular activities, even those responsible for control of gene function and regulation of gene expression. We also know that nearly all disease processes, even thoses due to trauma or infection, are caused by either disturbed gene function(s) and/or defective genetic predisposition, respectively. Thus, since protein function(s) are the final debouchment of all life processes in the cell, we can postulate, at least in theory, that the therapeutic use of proteins can replace for, and ameliorate, any disease condition caused by altered pathophysiological mechanisms, irrespective of whether they are secondary to genetic defects or induced by non-genetic disturbances.

Accordingly, hypotheses regarding the proper use of specific proteins to correct, and replace for, genetic defects can comprise all pathophysiological alterations resulting from nearly all underlying pathogenetic mechanisms. So, **structural and / or catalytic protein defects** that cause many disease conditions involving cell membrane or cytoskeleton integrity, cell transport mechanisms and chanellopathies, signal transduction defects and intracellular trafficking errors, major metabolic networks and minor metabolic circuits, for instance, could be corrected through supply of the needed protein in sufficient amounts and its proper localization in the cell. Similarly, **regulatory protein defects** that underlie the pathogenesis of many congenital malformations and, nearly, all known tumors could, also, be corrected either directly through gene therapy approaches involving gene transfer or, more efficiently, by supplying the normal protein to replace for the defective or deficient protein.

This reasoning underlies the basis of the endless list of research, experimental and applied trials aiming at disclosing the possibility and feasibility of the use of proteins in treatment of nearly all human diseases. These trials cover, almost, all known fields of pathogenesis of disease states including metabolic alterations, inflammation, carcinogenesis, apoptosis, teratogenesis, immune deficiency and autoimmunity.

Examples of some of these trials and indications include :

**1.** The use of proteins to **combat inflammation-associated illnesses, apoptosis and organ failure** through a strategy called **intracellular protein therapy** based on replenishing the intracellular stores of suppressor of cytokine signaling 3 protin (SOCS3) which plays a critical role in mediating signal transduction processes that effectively suppresses the devastating effects of acute inflammation (**Daewoong Jo et al., 2005**).

**2.** The use of specific synthetic fusion proteins capable of **arresting growth of refractory brain tumors** (**Liu Tie Fu et al., 2003**).

**3.** The use of recombinant surfactant associated proteins A and D (SP-A and SP-D) in attenuating inflammatory processes in neonatal chronic lung disease, cystic fibrosis, and emphysema (**H Clark and K Reid, 2003**).

**4.** The use of specific monoclonal antibodies against protein products of oncogenes to suppress their oncogenic potential and metastasis-promoting functions, e.g. use of the monoclonal antibody (mAb) (3E10 Fv antibody-mediated p53 protein) as a potent and effective treatment in reducing growth and progression of metastatic liver tumors (**James Hansen et al, 2007**).

**Enzyme replacement therapy**

Enzyme replacement therapy represents the logical therapeutic approach for genetic disorders resulting from deficient and / or defective production of specific enzymes needed to conduct specific biological functions. Many genetic disorders, notably metabolic errors, result from deficient production of specific enzymes necessary for conducting certain metabolic processes. This deficiency leads to a cascade of metabolic disturbances and, in most cases, a wide spectrum of drastic pathological consequences. The same occurs if a defective enzyme is produced due to the genetic defect (**Graw et al., 2006**).

Recombinant DNA technology has been used for synthesis of recombinant enzymes which can replace the deficient or defective enzyme(s) in these disorders. Indeed, the technique has proved its safety and efficiency, and currently many genetic diseases that result from enzyme deficiency or production of defective enzymes are successfully amenable to this therapeutic approach.

Prerequisites for this therapeutic approach necessitates thorough understanding of the molecular profile of the enzyme including its specificity in targeting the intended cells or sub-cellular organelles, its efficiency in conducting its metabolic function(s), its immunogenic properties and its possible toxic effects. Also, there are many requirements that should be met with to insure success of this therapeutic modality, e.g. **efficiency** of the enzyme, **stability** of the enzyme in order to insure a sustained function for a substantial period of time, **safety** of the enzyme and the feasibility of producing large quantities of the enzyme at a low affordable cost (**Hopkin et al., 2003**).

**Limitations of protein replacement therapy**

Like any other therapeutic modality, enzyme replacement therapy has its limitations in therapy. However many of these limitations have been overcome by various techniques, others, however, are still amenable for experimentation and research. These limitations are shared by other similar approaches including protein and hormone replacement, and include:

**1- The need for proper intracellular localization of the enzyme**

For many genetic disorders, transportation of the recombinant enzyme to inside the cell is necessary to conduct its functions. Normally, enzymes do not enter cells, they function either inside their cells of origin where they are synthesized, in the extra-cellular matrix or in the blood stream. Accordingly, delivery systems designed to enable transportation of the enzyme across the cell membrane have been invented. Among these are the various biochemical modification of the configuration of the enzyme that allow utilization of normal cellular transport mechanism to target the enzyme to its normal location within the cell. Such modifications have been used with recombinant β-Glucosidase used in the treatment of Gaucher disease to enable it to enter the lysosomes resulting in an effective treatment of the disease.

**2- The need to overcome immunogenicity of foreign proteins**

Being large complex proteins, enzymes represent a real immunogenic challenge when administered via parentral route. Modifications to overcome these obstacles have been implied with many enzymes, e.g. the modification of Adenosine Deaminase (ADA) by attaching an inert polymer (polyethylene glycol) to make the enzyme less immunogenic and, also, to improve its stability and extend its half life.

**3- The need to overcome the blood brain barrier**

This is essential for delivering enzymes involved in pathogenesis of inherited biochemical disorders of the brain. Modifications of the enzyme structure have to be done to enable its transport across the blood-brain barrier.

**4- The need to keep the enzyme stable and intact** during its transport without being inactivated till it arrives at its target cells or location.

**5- The need to design proper carriers** for transportation of the enzyme, for trans-membrane delivery and for proper intracellular localization.

**6- The need to design suitable animal test models** for preliminary studies and preclinical assessment.

**7- The need to establish the proper dosage and treatment protocols** for the enzyme replacement therapy (**Schilfman et al., 2001**).

As listed in table 2, it is apparent that many important genetic diseases are currently amenable to treatment with recombinant proteins, enzymes and hormones. Prominent in this list are treatment of **Gaucher disease Type 1** with recombinant β-gluosidase (**Weinreb et al., 2002**), treatment of **Fabry disease** with recombinant α-galatosidase A (**Lannou et al, 2001) and** treatment of **Pompe disease** with recombinant α-glucosidase (**Johanna M.P. et al., 2004**).

With more refining of the techniques and invention of more advanced techniques depending on genetic engineering and nanobiotechnology methods, more and more of genetic errors resulting from deficient or defective production of these biomolecules are expected, and are actually being experimented, to be enrolled in the list.

**Non-protein addition/replacement therapy**

As referred to previously in many sites, addition of other non-protein substrate contributes effectively to the comprehensive framework of therapy of genetic disorders. Non-protein substrates include a wide variety of active biomolecules like vitamins, minerals, trace element and neurotransmitters, in addition to the traditional use of carbohydrate and lipid substrates in dietary management of many inborn errors of metabolism.

Non-protein biomolecules exert their functions via different mechanisms. **Vitamins**, for instance, in large pharmacological or mega doses (megavitamin therapy) might act as potentiators of inefficient enzymes that depend on these vitamins as cofactors or prosthetic groups, like the use of large doses of Vitamin B6 in treatment

of many cases of homocystinuria due to cystathionine ß-synthase (CBS) deficiency and the use of thiamine supplementation in maple syrup urine disease. **Trace elements** likewise act by the same mechanism, but they have a wider range of effects because many of them share in the integral structure of many important regulatory molecules in the cell, like zinc incorporated in many antioxidant enzymes as well as in zinc fingers that act as transcription regulators and as interaction modules that bind DNA, RNA, proteins or small molecules, and copper which plays critical roles in keeping functional integrities of the basal ganglia, the cornea, the liver and the erythroid blood forming cells.

Carbohydrates and lipids are supplemented as part of dietary management of many metabolic diseases. Carbohydrates, for instance, are the mainstay of management of hypoglycemia of many glycogen storage diseases and fatty acid oxidation defects. Lipids also constitute a major source of nutritional supply of energy and specific components, like medium chain triglycerides or long-chain polyunsaturated fatty acids in many genetically-determined metabolic and nutritional disturbances (**Koletzko B et al., 2007**).

## 3. Organ, tissue, cell replacement and transplantation

**Transplantation therapy**, as a form of replacement therapy, is a wide term that encompasses too many varied applications. The concept of replacement therapy is a logic one based on the known pathogenetic mechanisms of most genetic disorders. Insufficient production of a gene product or its production in a defective way can be corrected by replacing the deficient or the defective product with a normal natural or synthesized one. This reasoning demarcated the narrow-scale use of the concept in hormone replacement therapy for endocrinal disorders, the use of immunoglobulin preparations for humoral immunodeficiency states, the use of anti-hemophilic globulin for hemophilia, the use of recombinant human enzyme preparations for many enzyme-deficient conditions, and similar allied disorders.

Extension of the concept of replacement therapy to a wide-scale applications resulted in a revolutionary turn in medical practice. Thus, a whole organ might be replaced as in **organ transplantation**. In **tissue transplantation**, a part of an organ is replaced with a healthy one like cardiac valve transplantation, cornea transplantation, and bone grafting. **Cell infusion** or replacement, like whole or fractionated blood transfusion or **stem cell therapy** is, in a sense, a transplantation technique. However, in stem cell transplantation the goal is to offer affected patients with a life-long chance for these cells to replace defective cells, damaged tissues, or failing organs.

Despite remarkable achievements in this field, chronic allograft rejection, the side effects of the long-term immunosuppressive treatment and organ shortage are still the major obstacles to achieving long-term survival. Precautionary measures needed to ensure success of replacement therapy stem mostly from fears of rejection of transplanted, infused or injected foreign material. However, advances in immunogenetics allowed for better prognoses by refining selection of compatible donors, and expected advances in genetic manipulation techniques aiming at prediction or detection of rejection at its earliest stages (**Sankaran et al., 1999**), nullifying foreign antigen processing and detection, and induction antigen-specific tolerance would surely lessen the risk of rejection and related complications to a minimum (**Gudmundsdottir and Turka, 1999** & **Jessamyn et al., 2002**).

One major complication facing organ transplant recipients is the requirement for life-long systemic immunosuppression to prevent rejection. The advent of immuno-suppressive drugs participated actively in ensuring the success of transplantation therapy, however, their use implies potential major side effects to exposed patients which are associated with an increased incidence of malignancy and susceptibility to opportunistic infections. Many gene therapy techniques have been advented to reduce and obviate these risks. Gene therapy has the potential to eliminate problems associated with immunosuppression by allowing the production of immunomodulatory proteins in the donor grafts resulting in local rather than systemic immunosuppression. Alternatively, gene therapy approaches could eliminate the requirement for general immunosuppression by allowing the induction of donor-specific tolerance. Gene therapy interventions may also be able to prevent graft damage owing to non-immune-mediated graft loss or injury and prevent chronic rejection (**Bagley and Iacomin, 2003**).

Another major challenge to transplantation approaches is failure of transplanted organ, tissue, or cells to maintain their own survival. Enhanced apoptosis of transplanted organs or tissues is a common complication of transplantation therapy. Furthermore, some of the immunosuppressive drugs currently in clinical use might exert their activity at least in part through effects on apoptotic pathways. From the available data, it can be inferred that apoptosis contributes to the outcome after organ transplantation, being involved both in graft rejection and in transplantation tolerance (**Kabelitz Dieter, 1998**). Many genetic therapies were tried to lessen and obviate this risk with promising success, for instance, the use of heme oxygenase-1 gene transfer to prolong survival of cardiac allograft (**Braudeau et al., 2004)** and use of anti-apoptotic genes for prolonging survival of pancreatic islets transplantation **(Contreras et al, 2001).**

**A- Organ transplantation**

**I. Bone marrow transplantation (BMT)**

Bone marrow transplantation, the treatment of choice for many genetic diseases, may be efficacious for three catagaroies of these diseases. The first is one in which the defect is expressed in the marrow stem cell, such as in svere combined immune-deficiency disease where virtually every child with the disease can be offered a curative bone marrow transplant (**Cowan et al, 1985**).

Other congenital marrow stem cell defects for which the procedure has been used include β-thalassemia major (**Chandy et al., 2001**), Wiskott-Aldrich disease, chronic granulomatous disease and osteopetrosis (**Hobbs, 1988**).

The second category of genetic diseases comprises those where the defect is expressed in all tissues but involves mostly the marrow stem cell population. For example, adenosine deaminase deficiency and nuclosidase phosphorylase deficiency, both of which results in severe combined immune-deficiency, and glucocerebrosidase deficiency or Guacher disease, are disorders that principally involves bone marrow-derived lymphocytes and monnuclear phagocytes, respectively. **BMT** with the successful replacement of these hematopoietic stem cells can significantly ameliorate the manifestation of these disorders (**Parkman, 1986**).

The third category of genetic diseases for which **BMT** may be indicated includes those in which the defect is expressed in all tissues with concomitant substantial systemic disease involving many organs. The rationale for a bone marrow transplant in these patients is dependent on marrow-derived tissue histiocytes including Kupffer and Ito cells in the liver, Langerhans cell in the skin, microglia cell in the central nervous system, osteoclasts in the bones and macrophages in the spleen, lymph nodes, tonsil, and peritoneum. These marrow derived cells of donor origin can repopulate organs in the recipient and provide a new source of activity.

The **lysosomal storage diseases** also belong to this category of genetic diseases. More than 100 children with various lysosomal storage diseases have received **BMT**. Nearly half of them were affected with Hurler disease due to α-L-iduronidase deficiency. BMT has been successful in lessening many of the manifestations of this disease and substantially altering its natural history (**Peters et al., 1996**). The dysmorphic facial and body features are ameliorated, joint function is improved as did the cardiopulmonary abnormalities. Amelioration of CNS manifestations has also been observed, hydrocephalus does not progress or, in some patients it does not develop, and in more than half of the patients there has been no further deterioration in mental state. Successful results of **BMT** in hurler disease paved the way for its use in many in other lysosomal storge disorders as well (**Hugh-Jones et al., 1984**).

Other indications for **BMT** in genetic diseases include:

1. Inherited metabolic disorders: Adrenoleukodystrophy, Hurler syndrome, metachromatic leukodystrophy and osteopetrosis.
2. Inherited immune-deficiency states and autoimmune disorders: Severe combined immunodeficiency, Wiskott-Aldrich syndrome, Chediak-Higashi syndrome.
3. Inherited red cell disorders: Pure red cell aplasia, sickle cell disease, β-thalassemia (**Chandy et al., 2001**).
4. Marrow failure states: severe aplastic anemia, Fanconi anemia, and others (**Thomas et al., 1972**).

**II. Kidney transplantation**

Kidney transplantation is the ultimate life saving treatment in patients with end-stage renal disease (ESRD). genetic diseases amenable to treatment with renal transplantation include polycystic kidney disease, renal dysplasia, fabry disease, autoimmune conditions like systemic lupus erythematosis and Good-Pasture's syndrome, and diabetes mellitus (DM) (**Brook and Nichlson, 2003**).

Clinical results, also, point to BMT as the treatment of choice in advanced Gaucher disease if an HLA-compatible donor is available (**Ringden et al., 1995**).

Successful renal transplantation in Fabry disease has been reported repeatedly. In addition to correcting the anemia, it also produces a marked improvement in other clinical manifestations of the disease (**Clement et al., 1982**).

**III. Liver transplantation**

Due to the countless dynamic physiological functions contributed by the liver to regulate, control and adjust most, if not all, the metabolic networks and metabolic circuits of all body organs, notably the CNS, the heart, the muscles, the intestine, the immune system, the skeletal system and the endocrine glands, even slight disturbances in liver functions could drastically affect the balance of the internal milieu needed to keep up life processes within optimal healthy standards. Control of blood ammonia and blood glucose levels, production of fatty acids as the major energy source for the heart, regulation of serum lipids, production of albumin and synthesis of coagulation factors are just few examples to site in order to illustrate the major role of the liver in maintaining life processes of the organism.

Affection of liver functions, and sometimes liver architecture, is quite common in genetic diseases. These functions might be incapacitated by genetic mutations underlying inborn metabolic errors. Similarly, congenital malformations of the liver, e.g. fibrosis or cystic disease, or of the biliary system, e.g. biliary stenosis or atresia,

could severely hamper liver functions. In these situations, unless alternative therapies are available or a liver transplant is offered, a fatal outcome ensues soon.

Inherited metabolic disorders leading to hepatic disease are proper candidates of liver transplantation if they fulfill the following specifications:

1. There is a specific hepatic enzyme deficiency that leads to acute or chronic liver failure and potential development of hepatic cancer like $\alpha_1$ antitrypsin deficiency, tyrosinemia type I, or glycogen storage disease type IV).
2. The precise metabolic defect is unknown but the main clinical features are attributable to hepatic dysfunction like Wilson's disease, Byler's disease and neonatal hemochromatosis.
3. The hepatic enzyme deficiency leads to acquired liver disease like Factor VIII and IX deficiency and subsequent chronic viral hepatitis.

The indications for transplantation in the first group are thus related not only to the correction of the metabolic defect but also to the expected severity of irreversible liver disease and prevention of hepatic malignancy. In disease such as alpha- 1- anti trypsin deficiency and Byler's disease which have predominantly hepatic manifestations with no effective medical treatment, liver transplantation provides both a phenotypic and functional cure (**Hood et al., 1980 & Soubran et al., 1990**).

Currently, indications of liver transplantation in genetic disorders include: glycogen storage disease types I, III, and IV (**Matern et al., 1999**), $\alpha_1$-antitrypsin deficiency (**Soubran et al., 1990**), ornithine trans-carbamylase deficiency and familial hypercholesterolemia. It is also indicated in children with inborn errors of metabolism due to primary hepatic enzyme deficiency that leads to liver disease and/or hepatic cancer, or severe extra hepatic disease, when complete resolution of the disease may be anticipated after transplantation. Cure of these metabolic diseases may require liver transplantation alone, or in combination with kidney, heart, or bone marrow transplantation (**Burdelski et al., 1991**). However, it must be noted that this approach is most effective if the transplant is done early. The transplantation procedure will seldom result in improvement of already existing skeletal and neurologic symptoms.

Until few years ago, tyrosinemia type I due to deficiency of the hepatic enzyme fumryl-acetoacetase leading to hepatic, renal, cardiac, and neurological disease and culminating in development of hepatocellular carcinoma by 2 years of age, has been a common indication for liver transplantation and there are many reports of how successful surgery has reversed the hepatic and extra hepatic manifestations of this disease despite persistent production of the toxic metabolite succinyl acetone by the kidneys (**Paradis et al., 1990 and Mieles et al., 1990**). However, the discovery of the chemical (NTBC) which prevents the formation of the toxic and carcinogenic metabolites and reverses the clinical and biochemical manifestations of this disease have altered the therapeutic approaches to this disease, and liver transplantation is seldom indicated, except for neglected cases, for treatment of the condition (**Lindstedt et al., 1992**),

In contrast, the main indication for transplantation in children with Wilson disease, in which early copper chelation by penicillamine may be effective, includes those who present with acute liver faliure and those in whom penicillamine treatment is ineffective (**Rela et al., 1993**). The reversal of the underlying abnormal copper metabolism has been reported after transplantation, as has the disappearance of Kayser Fleisher rings (**Song et al., 1992**) and neurological manifestations (**Mason et al., 1993**).

Neonatal hemochromatosis, which is a rare autosomal recessive disorder that presents with acute liver faliure in the first six weeks of life, is an indication for liver transplantation. Before the successful development of reduction hepatectomy and improvement in technical expertise, these children were considered too young and sick to be considered for liver replacement. Recent reports indicate complete resolution of the disease and good long term survival after transplantation (**Lund et al., 1993**).

Adequate medical treatment should prevent the need for liver transplantation in galactosemia (**Otto et al., 1989**) or glycogen storage disease, although the development of cirrhosis or malignancy in glycogen storage disease type IV may be an indication for transplantation (**Selby et al., 1993**).

In diseases with multi organ failure such as cystic fibrosis and proto-porphyria, liver transplantation may reverse the hepatic complications but has no influence on the extra hepatic manifestations (**Mieles et al., 1989 & Herbert et al., 1991**), and considerable care is required in selection of recipients. In children with severe cystic fibrosis with lung and liver disease, a heart/lung/liver transplant may be indicated.

Finally, liver transplant may be indicated for chronic viral hepatitis acquired with treatment for inherited disorders of hemostasis such as factor VIII and IX deficiency. Transplantation in this situation corrects not only the acquired liver disease but also the underlying inborn error of metabolism (**Delorme et al., 1990**). **Inherted metabolic disorders leading to extra hepatic disease and necessitating liver transplantation** include Crigler-Najjar type I, primary oxalosis, familial hypercholesterolemia, the urea cycle defects and propionic acidemia.

In Crigler-Najjar type I, urea cycle defects and propionic acidemia, medical treatment may not provide adequate metabolic control. There is a constant risk of acute metabolic deterioration leading to reduction in quality of life and an uncertain future with regard to mental development. Liver transplantation is indicated in those children with recurrent problems whose quality of life is unacceptable. The timing of the operation is important and should be performed before there is irreversible mental deterioration. Successful liver transplantation has been shown to correct the metabolic abnormalities of a number of urea cycle defects (**Todo et al., 1992**), propionic acidemia and Crigler-Najjar type I disease (**Kauffmann et al., 1986**).

In primary oxalosis, the situation is more complex, as the deficiency of the hepatic enzyme alanine-glyoxylate-amino transferase leads to renal failure and bone disease secondary to oxalate deposits. Successful management of the condition requires liver transplantation before the development of renal failure (**Watts et al., 1991**) and if renal failure is advanced, a combined renal and liver transplantation becomes mandatory (**Watts et al., 1991**).

Although plasmapharesis or medical treatment may control cholesterol concentration in heterozygotes with familial hypercholesterolemia, it is unlikely that this therapy will prevent the development of coronary artery disease in homozygote patients. As the metabolic defect is associated with relative deficiency of low density lipoprotein receptors on hepatocytes, liver transplantation successfully corrects the abnormal cholesterol metabolism (**Barbir et al., 1992**).

**IV. Heart transplantation**

Cardiac transplantation is the procedure by which the failing heart is replaced with another heart from a suitable donor. The procedure is generally reserved for patients with **end-stage heart failure** with a prognosis of less than a year to live without the transplant and who are not candidates for conventional medical therapy or have not been responsive to conventional medical therapy. It is a straightforward therapeutic intervention for genetic disorders of the heart which cripple its function to a stage incompatible with normal or tolerable life. **Complex congenital heart malformations** incompatible with life account for most of the indications for heart transplantation. Other indications include progressive **diseases of the myocardium** like hypertrophic or dilated cardiomyopathy, idiopathic cardiomyopathy, ischemic cardiomyopathy and intractable angina. **Malignant cardiac arrhythmias** for which conventional therapy has been exhausted and diseases with an ejection fraction of less than 20 % of the cardiac output are also absolute indications for heart transplantation (**Magee et al., 2004**).

**V. Lung transplantation**

This procedure has gained widespread acceptance as a therapeutic option for a diverse array of lung diseases, including both congenital and acquired, diseases. For patients with severe functional impairment and limited life expectancy, lung transplantation offers the possibility of a markedly improved quality of life and longer survival. Nonetheless, complications are frequent and result in constraints on long-term preservation of graft function and patient survival.

Indications of lung transplantation include chronic obstructive pulmonary disease including emphysema due to alpha$_1$-antitrypsin deficiency (**Hosenpud et al., 1998**), cystic fibrosis (**Oconnor and crystal, 2006**), idiopathic pulmonary fibrosis, primary pulmonary hypertension and Eisenmenger syndrome.

Less frequent indications of lung transplantation include sarcoidosis, lymphangio-leiomyomatosis, eosinophilic granuloma, drug-induced and radiation-induced pulmonary fibrosis and pulmonary disease arising from an underlying collagen vascular disorder. Although lung cancer has traditionally represented an absolute contraindication to transplantation, successful transplantation for broncho-alveolar carcinoma has been documented frequently (**Etienne et al., 1997**).

**VI. Pancreas transplantation**

This treatment modality involves implanting a healthy pancreas into a person who usually has diabetes mellitus. Because the pancreas is a vital multifunctioning organ, the recipient's native pancreas is left in place, and the donated pancreas is attached in a different location to make use of the potential of the recipient’s pancreas to mediate its endocrinal functions other than secreting insulin. In the event of rejection of the new pancreas which would quickly cause life-threatening diabetes, the recipient could not survive without the native pancreas still in place. The healthy pancreas comes from a donor who has just died or it may be a partial pancreas from a living donor (Type 1 cure- pancreas transplants). Whole pancreas transplants from living donors are not possible, again because the pancreas is a necessary organ for digestion. At present, pancreas transplants are usually performed in persons with insulin-dependent diabetes, who have severe complications that are usually of a renal nature (**Gruessner and Sutherland, 2005**).

Patients with pancreatic cancer are not eligible for pancreatic transplantation, since the condition has a very high mortality rate and the disease, being highly malignant, could and probably would soon return and invade the transplant.

**B- Tissue Transplantation**

While solid organs represent the dramatic and lifesaving aspect of donation after death, the transplantation of tissues from donors after death is a much larger-scale activity that benefits enormous numbers of patients, usually in a life-enhancing rather than a lifesaving manner. Some types of tissue transplantation, such as cornea transplantation and heart valve transplantation, have been established for many decades and are reasonably well understood by health professionals and the public. Many other types of tissue donation, such as bone, skin, tendons, etc. are much less well known but nonetheless result in beneficial treatment for large numbers of patients. Skin is used to prevent fluid loss and infection following a major burn, bone is used to improve the clinical success of a range of orthopedic operations, such as joint replacements, spinal fusions, and reconstructions following trauma or tumor.

**I. Cornea transplantation**

Cornea transplantation is one of the most successful tissue transplantation procedures due to the avascular nature of the cornea which obviates risks of rejection and the peculiar anatomical features of the cornea, e.g. easy access and use of local anesthesia. Cornea transplantation is indicated in some genetic diseases that cause progressive opacification due to progressive deposition of storage material in the cornea. These diseases include Fabry disease, $\mathbf{G_{M1}}$ generalized gangliosidosis, lecithin-cholesterol acyl-transferase (LCAT) deficiency (Norum or fish-eye disease), Tangier's disease, cystinosis, Hurler disease, mucolipidosis II (I-cell disease), mucolipidosis III, mannosidosis, galacto-sialidosis and multiple sulphatase deficiency (**Hoffmann et al 2002**).

Indications of transplantation in these diseases, however, are quite relative depending on many factors like recurrence of the pathology in the transplanted cornea in addition to much ethical debate regarding the feasibility of such treatment approach in patients with severe mental retardation.

**II. Heart valves transplantation or grafting**

This procedure is indicated for disease conditions impeding the function of the valves. Examples include congenital valve malformations or stenotic lesions that develop in the course of some diseases like Noonan syndrome and many collagen disorders.

**III. Bone grafting**

This technique might prove beneficial in supportive management of many genetic bone diseases. It can be used to correct varied types of skeletal malformations like correcting bone bending in campomelic dysplasia, stabilization of atlanto-axial subluxation in achondroplasia, lengthening short bones in severe short stature, replacement of deformed or diseases mandibular bones in some genetically-determined periodontal diseases (**Hochedlinger and Jaenisch, 2003**).

**C- Cell replacement/transfusion therapy**

Though a very effective and a most simple therapeutic approach, transfusion of cells is accompanied by many side effects that have to be anticipated and overcome for attaining optimal safety and feasibility of this treatment modality. These side effects include febrile reactions, transmission of viral (particularly cytomegalovirus) and rarely bacterial infections, fluid overload, graft versus host disease, hemolysis, and allo-immunization. Many advents and modifications, however, have been successfully tried to lessen the hazards of these side effects like testing for immune compatibility between the donor and the recipient to guard against serious, sometimes, fatal immunologically mediated reactions, proper storage conditions to lessen risk of pyrogenic reactions, pretesting for viral or bacterial contamination, proper dosage of transfused components and pre-storage leukodepletion to reduce the incidence of cytokine-mediated transfusion reactions and to decrease the transmission of cell-associated viruses such as CMV.

**I. Blood transfusion**

Replacement of deficient or defective cells by transfusion of normal immunologically compatible cells was one of the earliest applications of cell replacement approach. Whole or fractionated blood transfusion has been practiced since decades and is still keeping its title as the most vital lifesaving procedure in the history of medicine and surgery as well. Millions of lives have been saved owing to this approach including victims of trauma, patients in need of surgical intervention and patients with genetic disorders suffering from chronic hemolysis like the thalassemias.

Fractionated blood component transfusion is practiced on a wide scale routine for diseases of the immune system needing supply with efficient white blood cells to raise the immune potential of affected patients and also for disorders of blood platelets which are managed by platelet transfusion to obviate the hazards of bleeding tendency due to platelet defects or deficiency.

**II. Hepatocyte transplantation**

The liver is the most important site of protein synthesis and metabolism of exogenous and endogenous substrates. Thus, a large number of genetic diseases are expressed in the liver. Some of these defects produce liver disease (e.g., Wilson's disease or tyrosinemia). Others result in the extrahepatic expression of disease, e.g. hereditary oxalosis, factor IX deficiency and familial amyloidosis. Indeed, hepatocyte transplantation has already been

applied to a number of genetic conditions in which the mutation is expressed in the liver, including ornithine transcarbamylase deficiency (**Reyes et al., 1996**) and familial hypercholesterolemia caused by a deficiency of low-density lipoprotein (LDL) receptors (**Raper et al., 1996).**

A number of still unresolved issues will determine the usefulness of hepatocyte transplantation for the treatment of genetic diseases. First, the availability of livers for the isolation of hepatocytes. Second, it is not known what mass of hepatocytes is actually required for the treatment of inherited disorders. Third, it is not known how long the transplanted hepatocytes will continue to function. Moreover, it is uncertain whether additional enzyme activity could be gained by additional infusions of hepatocytes.

Hepatocyte transplantation has been tried, as a partial alternative to liver transplantation, in management of many genetic and non-genetic disorders of the liver. It proved partial success in correction of the metabolic defect of Crigler-Najjar syndrome type I by providing hepatic uridine di-phospho-glucuronate (UDP) glucuronyl-transferase, the enzyme that is defective in the syndrome, in amounts sufficient to produce the desired clinical benefit leading to reduction in the patient's serum bilirubin concentration and in the amount of bilirubin conjugates in bile, and the amount of phototherapy required to control the bilirubin concentrations (**Fox et al., 1998**).

Hepatocyte transplantation has also been explored as a mechanism for enhancing liver function in patients with acute liver failure due to many disease conditions including inborn errors of metabolism (**Strom et al., 1997 & Bilir et al., 1996**). Such treatment is attractive since, if given enough time, the liver can regenerate, and most patients recover essentially normal liver function. The capacity of the liver to recover from massive hepatic necrosis has been shown by the use of auxiliary orthotopic liver transplantation to treat fulminant hepatic failure. In as many as two thirds of the patients treated in this fashion, the native liver ultimately recovers function (**Neuhaus and Bechstein, 1997**). The fact that the infused hepatocytes in the patients continued to function for nine months is important, because it often takes months for the native liver to recover.

**III. Myoblast transplantation**

Myoblast transplantation is an attractive new therapeutic approach that revealed acceptable potency and success in many disease conditions at least on experimental and preliminary clinical trials. It has been used in treatment of ischemic cardiomyopathy (**Menasché et al., 2001**) and data of its safe use as an alternative to heart transplantation in cases with heart failure due to many disease conditions are being processed.

Similar, though guarded, success has also been achieved with histocompatible myoblast transplantation in dystrophin-deficient animal models, and accumulating results suggest that cell-based therapies, such as myoblast transfer therapy, are likely to become an integral part of any approach to treat myopathies such as Duchenne muscular dystrophy (**White Jason et al., 2001**).

**D- stem cell therapy**

Stem cells are cells that divide to form one daughter cell that goes on to differentiate, and one daughter cell that retains its stem-cell properties. All stem cells regardless of their source have three general properties: they are unspecialized, they are capable of dividing and renewing themselves for long periods, and under certain physiologic or experimental conditions, they can be induced to become cells with special functions, i.e. they can give rise to specialized cell types.

Stem cells are important for living organisms for many reasons. In the 3-to 5-day-old embryo, called a blastocyst, stem cells in developing tissues give rise to the multiple specialized cell types that make up the heart, lung, skin, and other tissues. In some adult tissues, such as bone marrow, muscle, and brain, discrete populations of adult stem cells generate replacements for cells that are lost through normal wear and tear, injury, or disease.

Research on stem cells aims at disclosing how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. This promising area of science is also leading scientists to investigate the possibility of cell-based therapies to treat disease, which is often referred to as **regenerative or reparative medicine**. It has been hypothesized that stem cells may, at some point in the future, become the basis for treating diseases such as Parkinson's disease, diabetes, and heart disease. As we learn more about stem cells, it may become possible to use the cells not just in cell-based therapies, but also for screening new drugs and toxins and understanding birth defects.

**Sources and types of stem cells**

**Embryonic stem cells**, as their name suggests, are derived from embryos. Specifically, embryonic stem cells are derived from embryos that develop from eggs that have been fertilized in-vitro in an in-vitro fertilization clinic and then donated for research purposes with informed consent of the donors. They are not derived from eggs fertilized in a woman's body. The embryos from which human embryonic stem cells are derived are typically four or five days old and are a hollow microscopic ball of cells called the blastocyst. The blastocyst includes three structures: the trophoblast, which is the layer of cells that surrounds the blastocyst; the blastocoel, which is the hollow cavity inside the blastocyst; and the inner cell mass, which is a group of approximately 30 cells at one end of the blastocoel.

Fetal development begins with totipotent cells which include the fertilized egg (The zygote) and the first 4 or so cells produced by its cleavage (as shown by the ability of mammals to produce identical twins, triplets, etc.). In mammals, totipotent cells have the potential to become any type in the adult body and/or any cell of the extra embryonic membranes (e.g. placenta). In mammals, the expression totipotent stem cells is a misnomer because these cells cannot make more of themselves.

**True fetal stem cells** comprise two cell types:

**1. Pluripotent stem cells**

These are true stem cells, with the potential to make any differentiated cell in the body, but cannot contribute to making the extraembryonic membranes (which are derived from the trophoblast). They include three types of cells:

**a. Embryonic Stem (ES) Cells**: these can be isolated from the inner cell mass (ICM) of the blastocyst, the stage of embryonic development when implantation occurs. For humans, excess embryos produced during in vitro fertilization (IVF) procedures are used. Harvesting ES cells from human blastocysts is controversial because it destroys the embryo, which could have been implanted to produce another baby (but often was simply going to be discarded).

**b. Embryonic Germ (EG) Cells**: these can be isolated from the precursor to the gonads in aborted fetuses.

**c. Embryonic Carcinoma (EC) Cells**: these can be isolated from teratocarcinomas, a tumor that occasionally occurs in a gonad of a fetus. Unlike the other two, they are usually **aneuploid**.

All three of these types of pluripotent stem cells can only be isolated from embryonic or fetal tissue and can be grown in culture, but only with special methods to prevent them from differentiating.

**2. Multipotent stem cells**

These are true stem cells but can only differentiate into a limited number of types. For example, the bone marrow contains multipotent stem cells that give rise to all the cells of the blood but not to other types of cells. Multipotent stem cells are found in adult animals, perhaps most organs in the body (e.g., brain, liver) contain them where they can replace dead or damaged cells. These adult stem cells may also be the cells that, when one accumulates sufficient mutations, produce a clone of cancer cells.

**Using Stem Cells for Human Therapy**

Many genetic and non-genetic diseases arise from damage to differentiated cells. Examples include: insulin-dependent diabetes mellitus (IDDM) where the beta cells of the pancreas have been destroyed by an autoimmune attack, Parkinson's disease where dopamine-secreting cells of the brain have been destroyed, spinal cord injuries leading to paralysis of the skeletal muscles, ischemic stroke where a blood clot in the brain has caused neurons to die from oxygen starvation, multiple sclerosis with its loss of myelin sheaths around axons and blindness caused by damage to the cornea.

The great developmental potential of stem cells has created intense research into enlisting them to aid in replacing the lost cells of such disorders. While some success has been achieved with laboratory animals, not much has yet been achieved with humans. One exception is the culturing of human epithelial stem cells and using their differentiated progeny to replace a damaged cornea. This works best when the stem cells are from the patient (e.g. from the other eye). Corneal cells from another person (an allograft) are always at risk of rejection by the recipient's immune system.

**Prospects of adult stem cells**

The wide distribution of adult stem cells in nearly all body organs has led scientists to ask whether adult stem cells could be used for transplants. In fact, adult blood forming stem cells from bone marrow have been used in transplants for 30 years. Certain kinds of adult stem cells seem to have the ability to differentiate into a number of different cell types, given the right conditions. If this differentiation of adult stem cells can be controlled in the laboratory, these cells may become the basis of therapies for many serious common diseases.

The history of research on adult stem cells began about 40 years ago. In the 1960s, researchers discovered that the bone marrow contains at least two kinds of stem cells. One population, called hematopoietic stem cells, forms all the types of blood cells in the body. A second population, called bone marrow stromal cells, was discovered a few years later. Stromal cells are a mixed cell population that generates bone, cartilage, fat, and fibrous connective tissue. Also in the 1960s, scientists who were studying rats discovered two regions of the brain that contained dividing cells, which become nerve cells. Despite these reports, most scientists believed that new nerve cells could not be generated in the adult brain. It was not until the 1990s that scientists agreed that the adult brain does contain stem cells that are able to generate the brain's three major cell types: astrocytes and oligodendrocytes, which are non-neuronal cells, and neurons, or nerve cells.

Perhaps the most important potential application of human stem cells is the generation of cells and tissues that could be used for cell-based therapies. Today, donated organs and tissues are often used to replace ailing or destroyed tissue, but the need for transplantable tissues and organs far outweighs the available supply. Stem cells,

directed to differentiate into specific cell types, offer the possibility of a renewable source of replacement cells and tissues to treat diseases including Parkinson's and Alzheimer's diseases, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, and rheumatoid arthritis.

For example, it may become possible to generate healthy heart muscle cells in the laboratory and then transplant those cells into patients with chronic heart disease. Preliminary research in mice and other animals indicates that bone marrow stem cells, transplanted into a damaged heart, can generate heart muscle cells and successfully repopulate the heart tissue. Other recent studies in cell culture systems indicate that it may be possible to direct the differentiation of embryonic stem cells or adult bone marrow cells into heart muscle cells.

In patients who suffer from type I diabetes, the cells of the pancreas that normally produce insulin are destroyed by the patient's own immune system. New studies indicate that it may be possible to direct the differentiation of human embryonic stem cells in cell culture to form insulin-producing cells that eventually could be used in transplantation therapy for diabetics.

To realize the promise of novel cell-based therapies for such pervasive and debilitating diseases, scientists must be able to easily and reproducibly manipulate stem cells so that they possess the necessary characteristics for successful differentiation, transplantation and engraftment. The following is a list of steps in successful cell-based treatments that scientists will have to learn to precisely control to bring such treatments to the clinic.

**The Production of Human Embryonic Stem Cells By Somatic-Cell Nuclear Transfer**

The production of human embryonic stem cells by somatic-cell nuclear transfer depends on a profound but obscure event that takes place when the nucleus of a "donor" somatic cell is injected into an enucleated ovum. Somehow, the cytoplasm of the oocyte reprograms the chromosomes of the somatic cell's nucleus so that the newly formed cell becomes pluripotent. The cell develops into a blastocyst from which embryonic stem cells can be derived that carry a set of chromosomes identical to that of the donor. The "tailored" embryonic stem cells thus derived have fueled hope for new treatments for degenerative diseases such as type 1 diabetes and Parkinson's disease. They are believed to be pluripotent that is, they can differentiate, under appropriate conditions, into cells of any type. With a nuclear complement that is identical to that found in the somatic-cell donor, they are unlikely to be rejected by that donor.

In a recent study, Cowan and colleagues tested the hypothesis that, like the oocyte's cytoplasm, the human embryonic stem cell can also reprogram the chromosomes of a somatic cell. They encouraged the fusion of fibroblasts and embryonic stem cells by coculturing cells of both types in an agent that facilitates membrane fusion, and they obtained stable tetra-ploid hybrid cells, each of which had a single nucleus. These cells looked and behaved like embryonic stem cells. For example, a protein characteristic of embryonic stem cells was expressed from RNA transcribed from a fibroblast chromosome; the cells seemed to be immortal (they have been passaged more than 50 times). They developed and differentiated into embryoid bodies (in vitro) and teratomas (in vivo) each of these had tissues expressing markers characteristic of each of the three germ-cell layers (endoderm, mesoderm, and ectoderm). Thus, the hypothesis would seem to be correct: human embryonic stem cells can reprogram adult somatic-cell chromosomes after cell fusion, a revolutionary finding that could alter all current concepts of many therapeutic approaches to genetic disorders.

Stem cell transplants could be used to treat a wide variety of diseases, especially those affecting specific cells types such as cardio-myocytes, dopaminergic neurons, and islet β cells that have been destroyed. There is anecdotal evidence that patients with myocardial infarction recover faster when autologous HSCs are injected directly into the heart. Fetal midbrain is currently being used in a trial setting in the treatment of Parkinson’s disease, and pancreatic duct cells are being used in the treatment of diabetes.

Cancer is another area where manipulation of stem cells is vital. Several methods have been used, including the introduction of suicide genes into malignant stem cells. Examples of this are herpes simplex thymidine kinase and/or cytosine deaminase, which could be used in combination with oncolytic viruses that could theoretically be able to reach and destroy widespread metastases (**Brenner, 1996** & **Anderson, 2000**).

**Cord blood transplantation** is a form of cell therapy that involves use of characterized mature cells, embryonic stem cells or umbilical cord blood cells as alternative treatment approaches with many advantages like increased tolerance to histoincompatible donor blood cells, decreased risk of graft versus host disease, and wide availability of placental blood. It has been used in more than 2000 patients with malignant or non-malignant disorders.

**Hemopoietic stem cell transplantation** is the single most useful entity for management of genetic disorders. The transplant can be a source of the missing protein or replace a damaged organ. When a transplant is used as a source of a deficient protein it is important that the protein reaches target tissue. Transplant therapy can be effective for selected metabolic diseases like storage disorders (MPS I, VI, VII, metachromatic leukodystrophy, globoid cell leukodystrophy, X-linked adrenoleukodystrophy, fucosidosis, mannosidosis, Gauchers disease, Niemann Pick disease type B and malignant infantile osteopetrosis (**Peters et al., 1996** & **Chandy et al., 2001**).

Hemopoietic cell transplants have been used for their additional ability to generate a specific immunological response in the recipient. The proliferating lymphoid cells derived from the donor stem cells have been used to seek out and destroy remaining tumor cells (graft versus leukemia effect) and also to clear host cells of persisting viral infections, such as Epstein Barr virus. Elucidating the crucial differences in the subsets of lymphocytes responsible for the graft versus leukemia effect and graft versus host disease is currently the subject of much research.

**Embryonic stem cell transplantation** has brought an evolutionary change in genetics. Stem cells are self-renewing cells that can proliferate to make differentiated cell types of a tissue in vivo and continue self renewal for lifetime. Embryonic stem cells can give rise to the whole organism (Reproductive cloning) and non-reproductive or therapeutic cloning can provide stem cells for research and therapy. Embryonic stem cell therapy offers a valuable means of obtaining autologous cells for a variety of diseases. The cells are genetically identical to the patient's cells, so the risk of immune rejection and need for immunosuppression is eliminated. Therapy can be repeated whenever needed.

**Pluripotent embryonic stem cells** or multi potent adult stem cells have also been used for therapeutic cloning, however with much ethical debate and practical limitations. Therapeutic potential of adult stem cells is much lower than embryonic stem cells. Stem cell and progenitor cells of the bone marrow and peripheral blood have been used in many situations, e.g. for repair of cardiac tissue after acute myocardial infarction, neuro-degenerative diseases, amyotrophic lateral sclerosis and periodontal diseases (**Hochedlinger and Jaenisch, 2003**).

## 4- Pharmacological Therapy

### General guidelines of drug action and pharmacotherapy

Like most other human diseases, pharmacological or drug therapy represents, by far, the most commonly used approach in management of genetic diseases. The potential of drug use in genetic errors stands on the same theoretical hypotheses underlying their use in other disease states: disturbed pathophysiological alterations which mediate pathogenesis of disease complications can be arrested, modified, or even reversed by pharmacological agents. The specific nature of the pathogenetic mechanisms that underlie development and progression of genetic disorders make them liable for manipulation by pharmaceuticals in view of the multiple functional levels that can be targeted.

So, starting with the first stage of gene function, drugs could act as enhancers or silencers to increase or decrease gene expression, respectively. Further, they might be able to cross over stop or non-sense mutations of the gene. This effect has been observed upon using the aminoglycoside gentamycin in therapeutic trials in patients with Hurler disease and cystic fibrosis where regain of gene function and synthesis of the missing gene products, α L-iduronidase and trans membrane conductance regulator respectively, with concomitant biochemical alteration was observed (**Joan Stephenson, 2001**). Another example of drug effects on gene function was disclosed upon finding that the antidepressant, Imipramine, acts by targeting many other points along the process of gene function and protein synthesis including cAMP metabolism, synaptic function, and protein processing by serine proteases (**Juha Knuuttila et al., 2004).**

Current research concentrates on the possibility of using drugs to manipulate other stages of gene functions including transcription regulation, splicing modification, up regulating or down regulating translation and alteration of post-translational modifications. The current use of pharmacological synthetic chaperones to correct folding errors in synthesized proteins exemplifies the great potential of drugs in therapy of genetic diseases once the underlying pathogenetic mechanism is identified and the proper pharmaceutical preparation is designed. Actually, the study of metabolic responses to drugs, environmental changes and diseases constitutes the core components of a new branch shared by genomics, biochemistry, physiology, medicine and pharmacology called **Metabonomics.** Prospects for advances in Metabonomics can usher for a new era of treatment of disease based on regulating molecular processes involved in pathogenesis, irrespective of the nature, etiology, or pathology of the disease.

Drugs or pharmaceutical agents exert their beneficial, or drastic, effects through interaction within the frame of specific metabolic networks or metabolic circuits with other biomolecules, substrates, metabolic intermediates or end stage metabolites in the cell. These effects are mediated through varied pathways dependent on the nature, specificity, drug kinetics and reaction dynamics. These pathways conducted and executed via different mechanisms notably: **diversion**, **depletion**, **inhibition** and **activation**.

The ways how drugs act are countless in view of the large number of possible reaction models that describe interactions between active molecules. Many of these models, however, have been disclosed and they include different mechanisms based on the following theoretical hypotheses:

1. The drug may exert a **direct inactivating action** on a cellular organelle, a macromolecule or an enzyme thus arresting initiation or progression of harmful pathological alterations.

2. The drug may **activate and enhance the potential of a metabolic reaction** by activating one or more of the enzymes involved in mediating the reaction.
3. The drug may **selectively combine with harmful or toxic materials** or metabolites accumulating in the cells or tissues thus removing the harm and reducing the burden of the disease.
4. The drug may act synergistically or may interfere with cellular and sub-cellular regulatory mechanisms including gene expression, protein synthesis, signal transduction or pore chanell functions of the cell membrane thus affecting many, or all, consequences of these regulations.

Thorough understanding and complete awareness of the whole aspect of drug function(s) is mandatory and of prime importance before drug prescription for genetic, or non-genetic, diseases. It must be always kept in mind that a drug might exert a required beneficial therapeutic effect by affecting one or more metabolic pathway with concomitant occurrence of unwanted, sometimes serious or fatal, side effects due to its effect on other pathways. Accordingly, drug therapy must be dealt with cautiously and preliminary and follow-up investigations to predict possible side effects must be a part of routine drug therapy of all diseases including genetic diseases. These guidelines also apply for cases requiring multidrug management which is quite common in genetic states due to the chronic lifelong nature of these errors. Drug interactions might cause serious and fatal sequels, and strict **drug management protocols** for both single and multidrug therapy in these patients must be constructed and formulated and care givers must adhere to these protocols quite rigidly without any inattention.

**Mechanisms of drug action and pharmacodynamics**

**I. Diversion**

Diversion therapy implies enhanced use of alternative metabolic pathway to reduce the concentration of blocked substrates or harmful metabolites. A prominent example of diversion therapy is the use of synthetic enzyme Phenylalanine ammonialyase (PAL) as a substitute for the enzyme phenylalanine mono-oxygenase which is deficient in PKU. PAL diverts the metabolism of excess phenylalanine converting it to metabolically insignificant amounts of ammonia and trans-cinnamic acid, a harmless metabolite. the latter is converted to benzoic acid and rapidly excreted in urine as hippurate leading to palpable reduction in plasma phenylalanine levels in PKU patients (**Sarkissian et al., 1999 & Sarkissian and Gámez, 2005).**

A similar approach has been successful in helping to reduce the cholesterol level in heterozygotes for familial hypercholesterolemia by the **diversion of an increased fraction of cholesterol to bile acids synthesis** by oral administration of non-absorbable resins such as cholestyramine, which bind bile acids in the intestine and increase their fecal excreation. The normal allele of these patients is thus stimulated to produce more hepatic receptors for LDL-bound cholesterol with consequent increase in LDL receptor-mediated uptake of cholesterol by the liver resulting in significant reduction in plasma cholesterol level. This example illustrates clearly an important principle: autsomal dominant diseases may sometimes be treated or alleviated by increasing the expression of the normal allele (**Goldstein et al., 2001**).

**II. Depletion**

Depletion, a sort of removal therapy, refers to removal of accumulating harmful metabolites or toxic compounds, which account for most of the deleterious consequences of metabolic and storage disorders.

A successful application of depletion therapy is in the treatment of urea cycle disorders where accumulating ammonia can be reduced to normal levels by administration of sodium benzoate which binds it with glycine to form hippurate, which is excreted in the urine (**O'connor and Crystal, 2006**).

Depletion is, also, routinely practiced on a wide scale since long time in management of thalassemia and other chronic hemolytic anemias by using the iron chelator Desferoxamine to get rid of the excess iron overload in tissues and organs of these patients. Similarly, the copper chelators D-Pencillamine and Trientene are used to reduce high levels of copper in patients with Wilson disease (**Roberts and Schilsky, 2003**).

Depletion therapy approach has been tried in animal models with promising results as regards cancer therapy. It has been found that in murine models with non-Hodgkin lymphoma, depletion of polyamines, important to tumor nutrition, by the plant lectin phytohaemagglutinin resulted in marked reduction in tumor growth and progression. These observations suggest that lectins, which exhibit growth-promoting effects on the gut, may have interesting applications in the formulation of new approaches with respect to cancer treatment (**Pryme Ian and Bardocz, 2001**).

**III. Inhibition**

Inhibition therapy implies the use of drugs to inhibit the occurrence or progression of certain reactions or functions within the body. Inhibition might be direct or indirect. Direct inhibition may be competitive due to structural similarities between the drug and the substrate in question, or it may be non-competitive inhibition depending on factors other than structural similarity. Indirect inhibition refers to inhibition exerted on an intermediate substrate or reaction which subsequently affects the target molecule or reaction in a cascade manner.

**Direct inhibition** is exemplified by the use of the inhibitory effect of Nitisinone (NTBC) on the enzyme 4-hydroxyphenylpyruvate dioxygenase as a complementary step in management of tyrosinemia type I. NTCB prevents the formation of fumarylacetoacetate from tyrosine and blocks the accumulation of the toxic metabolites thus reducing their toxic effects on body organs and tissues (**Koelink et al., 2006**).

Nitisinone (NTBC) has also been used in patients with Alkaptonuria as an inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase, which mediates formation of homogentisic acid. Urinary homogentisate excretion was markedly reduced, but safety of prolonged use of the drug has not been settled yet.

The inhibition of cholesterol synthesis through inhibiting the function of the enzyme (HMG-CoA reductase) or hydroxyl-methyl-glutaryl-CoA reductase by the statin class of drugs illustrates the concept of **competitive inhibition**. The statins are structurally similar to hydroxyl-methyl-glutaryl-CoA (HMG-CoA), a precursor of cholesterol, thus acting as competitive inhibitors of HMG-CoA reductase, the last regulated step in the synthesis of cholesterol (**Mabuchi et al., 1981**). Through this effect, they lower serum LDL cholesterol concentrations by up-regulating LDL-receptor activity as well as reducing the entry of LDL into the circulation (**Davignon et al., 1992**).

The pharmacological inhibition of enzymes is sometimes used to modify the metabolic abnormalities of inborn errors. Treatment of familial hypercholesterolemia clearly illustrates this principle. When the cholesterol load is increased by diverting it to other compounds or by removing it with physical methods, the liver tries to compensate for the decreased cholesterol intake by up- regulating cholesterol synthesis. Consequently, the treatment of familial hypercholesterolemia heterozygote is more effective if diversion therapy using cholestyramine resins is combined simultaneously with inhibition therapy aiming at reducing hepatic cholesterol synthesis by a statin class of drugs (**Goldestein et al., 2001**).

A significant contribution to inhibition therapy that might have widescale future implications in management of metabolic diseases was the finding that treatment of patients with Gaucher disease with the pharmaceutical agent (**NB-DNJ**) N-butyl-deoxy-nojirimycin, which is a potent inhibitor of the enzyme glucosyl-ceramide synthase (**UGCG**) resulted in decreased liver and spleen volumes and clinical improvement. Bone involvement and platelet and hemoglobin levels remained stable and the treatment was well tolerated although 24 months of clinical trial (**Pastores et al., 2005**).

This last finding, which seems to be a breakthrough research, will pave the way for the discovery of alternative drug therapy approaches for enzyme deficiency states for which no replacement enzyme is available or where targeting of the replacement enzyme is too improbable due to cellular localization of the pathology. It will also have great financial implications in view of the enormous costs of recombinant enzyme synthesis and application.

Enzyme inhibition by drugs bears great promises in treatment of genetic diseases caused by gene overexpression leading to over production of enzymes and enhanced metabolic pathways conducted and regulated by these enzymes. This is the case in cancer cells characterized by over production and over expression of enzymes that mediate all aspects of the malignant phenotype. Enzyme inhibition therapy is a quite effective approach in cancer treatment via inhibiting these enzymes. Inhibition of enzymes responsible for nucleic acid synthesis could force malignant growth to a standstill. Similarly, inhibition of enzymes responsible for angiogenesis would hasten apoptosis and forced necrosis of solid tumors. The list of possible therapeutic interventions through this inhibition concept in cancer is limitless and a considerable portion of current chemotherapeutic agents actually function via this mechanism.

**IV. Activation**

Activation of metabolic reactions and physiological functions by drugs is well known effect of many drugs and has been used since long time for therapeutic purposes. A drug may activate a reaction by direct activation of one or more of the enzymes mediating the reaction. It might, also, activate a reaction by inhibiting or inactivating other intermediate substrates that negatively affect the balance of the reaction, or may do the reverse, i.e. it may potentiate substrates that positively affect the dynamics of the reaction. These variable effects on agonists and/or antagonists constitute a major effect that contributes to the therapeutic actions of drugs. Other mechanisms of drug actions involve effects on other metabolic mediators, e.g. membrane receptors where binding of the drug to a certain receptor triggers a response similar to that elicited by active biomolecules, e.g. hormones.

Also, the ability of many drugs to interfere with normal intracellular and intercellular metabolic pathways, for instance interfering with signal transduction pathways, underlies the potent effects of drugs in therapy of diseases due to or dependent on these pathways, e.g. antiepileptic drugs that cause interruption of signal transduction-induced membrane depolarization-hyperpolarization states leading to arrest of impulse transmission along the axons and across the synapses in seizure conditions.

Still, other drugs act by more sophisticated mechanisms, e.g. the antiepileptic drug Valproic acid acts by inhibiting histone deacetylator enzymes which are involved in chromatin remodeling and regulation of transcription. The versatility of drug actions, however, is not a mercy in all times, it might be the cause of much

interference between different metabolic pathways with consequent adverse effects. In fact, the teratogenic effects of Valproic acid are attributed to its global indifferent effect(s) on the aforementioned enzymes and its effects on chromatin configuration.

Common examples of activation effects of drugs in metabolic circuits include the use of Phenobarbital to enhance the activity of glucoronyl transferase enzyme in order to enhance hepatic metabolism of bilirubin and reduce its level in states of hyper-bilirubinemia like Gilbert syndrome and Crigler-Najjar syndromes (**Wolkoff et al., 1978 & Crawford et al., 1988**). Residual enzyme must be present in order to achieve induction and enhancement of the metabolic effect by the drug. In diseases with total absence of the enzyme no such effects are observed. This finding can, sometimes, be used to differentiate between different phenotypes of the same disease. For instance, response to Phenobarbital is used to differentiate between the responsive type II with residual enzyme activity and the unresponsive type I with no such activity (**Sinaasappel and Jansen, 1991**).

Enzyme induction approach bears promising prospects in treatment of genetic diseases due to deficient production of enzymes, like many inborn errors of metabolism, and those due to enzyme-dependent enhancement of pathophysiological alterations, notably malignant diseases. Oncogene overexpression and over production of oncoproteins, many of them are enzymes mediating metabolic and metastatic aspects of malignant cells, is a constant finding in cancer. Genetic amplification, another constant finding in cancer cells, renders trials to inhibit this overproduction nearly fruitless and induction of enzymes that can drastically affect the malignant phenotype seems to be a more reasonable approach. For example, induction of enzymes mediating apoptosis could be a potent approach in arresting and fighting cancer. Induction of enzymes involved in metabolism of chemotherapeutic agents represents another promise in cancer therapy and induction of enzymes and pathways conducting immune responses to malignant cells represents, still further, another potent approach in the same direction.

**Vitamin therapy**

Though vitamins are not drugs in the strict sense, they are widely used as effective pharmaceutical agents in many genetic diseases. Being common constituents of most enzymes, either as co-enzymes, co-factors or as prosthetic groups, vitamins in pharmacological doses have a remarkable activating effect on many enzymes. This relationship explains the use of vitamins in many inborn errors of metabolism to activate enzyme potential of residual enzymes and correct some of the metabolic defects in these diseases. Examples include use of vitamin B1 (**Thiamine**) in maple syrup urine disease, use of vitamin B3 (**Niacin**) as an established treatment for elevated cholesterol and triglycerides, use of vitamin B6 (**Pyridoxine**) in patients with homocystinuria with B6-responsive Cystathionine β-synthetase deficiency, use of vitamin B12 (**Cobalamin**) for treatment of pernicious anemia, homocystinuria and methylmalonic aciduria and use of folic acid as a prophylactic medication against many birth defects notably of neural origin.

The known **antioxidant effect** of some vitamins constitute another vital aspect of vitamin functions and underlies their conventional therapeutic use in many genetic disorders attributable to damage caused by oxidant stress generated within deranged metabolic pathways. For instance, **vitamin E** (α-Tocopherol) supplementation is used to cure patients with the rare autosomal recessive Ataxia with Vitamin E deficiency due to mutation in the alpha tocopherol transfer protein gene (TTPA) (**Jayaram et al, 2005**). Vitamin E is used also in many other genetically-determined diseases where antioxidant stress due to free radical formation plays a part in their pathogenesis like atherosclerosis, multiple or disseminating sclerosis, cholestasis, Huntington disease and Alzheimer dementia. Also, v**itamin C**, up to 1 g/d, is recommended for older children and adults with Alkaptonuria. The mild antioxidant nature of ascorbic acid helps to retard the process of conversion of homogentisate to the polymeric material that is deposited in cartilaginous tissues, thus reducing the age-dependent complications of osteoarthritis and joint damage in these patients.

**Trace element therapy**

Trace elements play a vital role in most metabolic processes maintaining integrity of many important body systems and regulating inter-cellular adaptation. Information regarding role of trace elements in health is accumulating rapidly and is expected to be translated into applied use of these elements in pharmacological doses to complement management of many genetic diseases. The number of known trace elements that are essential for optimal health conditions in human is large and includes: Aluminum, Calcium, Chromium, Cobalt, Copper, Fluorine, Iodine, Iron, Lithium, Magnesium, Manganese, Cobalt, Phosphorus, Potassium, Selenium, Sulfur, Vanadium, Zinc and Nickel.

**Zinc**, for instance, is necessary for a healthy immune system, and is also of use in fighting skin problems such as acne, boils and sore throats. It is further needed for cell division, and is needed for integrity of the tissue of the hair, nails and skin. Zinc is further used in the growth and maintenance of muscles, is required for the synthesis of protein and collagen and is important to achieve normal growth and sexual development in children. Currently,

Zinc is used to raise immunity in patients with known immunodeficiency states like Down syndrome patients due to its role in immune competence and its antioxidant effects as well.

**Selenium** is an essential trace element that occurs in proteins in the form of seleno-cysteine. Selenium has an impressive antioxidant potential, and it works well with vitamin E to scavenge free radicals, helping to prevent gene mutation, cancer and the effects of aging. It is used for delaying premature aging, for protection against cardiovascular disease, in multiple sclerosis, sexual dysfunction, menopause and skin ailments**.**

## 5- Surgical intervention

**Surgical intervention** for treatment of birth defects and possible developing defects in genetic disorders plays a very important role in management of these diseases. Without such an approach, the life of patients with surgically-correctable congenital malformations would have been a real misery. The same guidelines applied for these procedures in non-genetic diseases apply also for genetic diseases. The list of congenital defects amenable for surgical correction either for radical cure or for alleviation of sufferings and prophylaxis against worse downhill progression is too long, examples of candidate conditions include congenital heart diseases, congenital urinary tract obstructions, congenital neural tube defects, congenital bone dysplasia and malformations, congenital defects of external genital organs, and many congenital ocular and auditory malformations.

**Fine surgical intervention** is, also, practiced for many genetic defects with considerable success. For instance, treatment of genetically-determined intractable seizure foci by bipolar electro-coagulation of functional cortex (**Guoming Luan et al., 2001),** use of isolated or combined fine **laser treatment** for ocular visual defects and dysplastic conditions of the cornea, the retina and the choroids, implantation of smart microchips for peripheral vaso-occlusive and myocardial diseases, and the use of **robotic surgery** for more safe and effective surgical management of many malformation disorders, reveal the role of fine or micro-surgical procedures in this therapeutic approach.

Surgical intervention in genetic diseases comprises the whole spectrum of severity of malformations ranging from mild easily managed defects, like cleft lip or undescended testes, to severe anomalies involving major organs, like complex congenital heart malformations. Examples of common congenital malformations amenable to cure by surgical intervention include: cleft lip and cleft palate, congenital pyloric stenosis, congenital dislocation of the hip, undescended testis, hypospadias, syndactyly, hydrocele and umbilical hernia (**Czeizel et al., 2002**).

Indications of surgical intervention in genetic disorders are determined by many factors including pathological nature of the lesion, its localization, its severity, its liability for recurrence, its effects on patient' performance, and health and personal factors related to age, weight, mental status and presence of other anomalies, among many others. These indications clearly delineate three distinct possible modalities of surgical treatment of genetic anomalies:

1. **Curative surgical intervention** when an anomaly is successfully removed or completely corrected, e.g. surgical treatment of congenital pyloric stenosis or spleenectomy in spherocytosis,
2. **Palliative surgical intervention** when progression of a congenital malformation is arrested or when its pathophysiological consequences are ameliorated by surgery, e.g. treatment of hydrocephaly by shunt operation.
3. **Prophylactic surgical intervention** when removal of a lesion obviates the affected patients the expected drastic complications of the lesion, e.g. subtotal colectomy with ileo-rectal anastomosis for patients with familial adenomatous polyposis syndrome.

In view of the amazing over progressing advances in surgical procedures and techniques, medical geneticists taking care of patients with congenital malformations are ethically and professionally obliged to follow these advances meticulously in order not to miss a chance of cure or palliation for their patients. For nearly all malformations at least some palliation could be offered by surgical intervention even for those which look as hopeless untouchable anomalies. Hypoplastic left heart syndrome, though uncorrectable lethal anomaly if left without intervention, could be cured by heart transplantation or alleviated by a series of operations done in stages. Congenital malformations of brain vasculature could, and almost, result in death or severe neurological defects if left untouched. Curative or prophylactic intervention of these malformations is currently possible with the recent advances in laser and fine robotic neurosurgical procedures.

## 6- Fetal Therapy

Fetal therapy implies therapeutic approaches aiming at treatment of fetal diseases before birth. Fetal therapy is a relatively old therapeutic practice. Attempts to treat and reverse the abnormalities of fetal hemolytic disease came in 1960 (**Liley, 1963**) and intra-peritonial transfusion used initially in 1970 had become a common form of intervention. In the late seventies and through eighties, attempts at surgical intervention of fetal diseases, such as shunting obstructed bladders and hydrocephalus, and the first trials of open fetal surgery, had been made (**Evans et al., 1990**). Some of the most important advances in fetal therapy have been achieved via pharmaceutical drugs, and the future of fetal therapy will surely include correction of genetic defects via gene therapy techniques (**Evans et al., 1992**).

**Fetal therapy** represents a crucial approach to avoid progression of inherited, or acquired, fetal diseases or malformations to a hopeless situation if left without intervention till birth. Fetal therapy approaches comprise both medical treatment and surgical procedures. **Medical drug treatment** of many fetal genetic diseases is possible for a number of conditions through delivering the medication to the fetus either by maternal administration or by direct fetal approach. **Surgical intrauterine management** of some fetal conditions, either via open fetal surgery or minimally-invasive fetoscopic surgery, has an important role in treatment of many fetal malformations. Intervention in hydrocephaly, many types of spina bifida, congenital diaphragmatic hernia, urinary tract obstruction and some congenital heart defects illustrates few applications of this approach in fetal therapy.

**1. Fetal drug therapy**

**Transplacental passage of substances**

Until recently, the only approach to the pharmaceutical treatment of an affected fetus was through the transplacental routs. With the introduction of high-resolution ultrasonography, the possibility of by-passing the placenta through direct invasive intra-amniotic, fetal intramuscular or fetal intravenous administration has been realized and is being increasingly evaluated (**Gembruch et al., 1988**). The principal advantages of direct targeting of the fetus are avoidance of maternal toxicity and the metabolic effects of administered agents, and the obviation of concern about the lack of placental permeability and transfer. It has been shown, for example, that the passage of cardiac agents across the placenta is less efficient in hydropic states, thus rendering the sickest vulnerable fetuses least likely to benefit (**Younis and Granat, 1987**).

Fetal drug therapy and prophylaxis has a pivotal role in treatment and/or prevention of a large number of genetic fetal disorders. Examples of such applications include use of corticosteroids to prevent masculinization of external genitalia in female fetuses with 21-hydroxylase deficiency syndrome (**Cerame et al., 1999)**, biochemical amelioration of methylmalonic aciduria and biotin-responsive multiple carboxylase deficiency and treatment of fetal goitrous hypothyroidism with L-Thyroxine (**H. Hashimoto et al., 2006**).

The correction of cardiac arrhythmias has become relatively commonplace with Amiodarone treatment (**Janette Strasburger et al., 2004)**, and reduction in the risks of neural tube defects is now possible with the use of pre-conceptual and early conceptual folic acid supplementations by the mother. Similarly, fetal lung functions can be enhanced by using a number of agents that increasing lung maturity, corticosteroids are the most commonly used agents, and a number of other agents have also been tried. The most common route of administering pharmaceutical agents is through the mother and the placenta, although the direct administration of certain agents is becoming more common (**Evans et al., 1993**).

**Congenital adrenal hyperplasia**

The fetal adrenal glands can be suppressed by dexamethasone taken by the mother. In congenital adrenal hyperplasia caused by 21-hydroxylase deficiency, there is impairment in the metabolic pathway from cholesterol to cortisol that creates both an excess of the metabolic intermediary 17- hydroxyprogesterone and a deficiency of cortisol. The excess 17-hydroxyprogesterone is metabolized by an alternative pathway producing excessive androstenedione and other adrenal androgens. The absence of cortisol results in a lack of feedback inhibition of the hypothalamus, and high levels of corticotropin (ACTH) are produced that stimulate further activity of the adrenal gland and exacerbation of 17-Hydroxyprogesterone excess, leading to more adrenal androgen production that induce virilization and musculinization of affected female fetuses (**Evans et al., 1985**). Following prenatal diagnosis, effective treatment of this condition is attained via maternal oral administration of 0.5 mg of dexamethasone three times a day at the very beginning of the 8th week of gestation (**Stephan et al., 1994**).

**Methylmalonic aciduria**

Methylmalonic aciduria is related to a functional vitamin B12 deficiency. B12 is required for the conversion of methylmalonyl CoA to succinyl CoA. Genetically determined causes of methylmalonic aciduria include defects in methylmalonyl-CoA mutase or in the metabolism of vitamin B12 to the co-enzymatically active form, adenocyanocobalamin, by the converting enzyme. Some patients may respond to administration of large doses of B12, which can enhance the amount of active holoenzyme (mutase apoenzyme plus adeno-cyano-cobalamin) (**Ampola et al., 1975**). This disease in the fetus is known to be associated with increased methylmalonic acid excretion in maternal urine.

Initial efforts to treat fetuses by high maternal oral vitamin B12 intake resulted in marginal decrease of maternal urinary methylmalonic acid excretion. When the intravenous administration of 5 mg of cyanocobalamin per day was introduced, however, there was rise in maternal serum B12 levels to more than six fold over normal, accompanied by a progressive decrease in maternal urinary methylmalonic acid excretion. In fact, maternal urinary methylmalonate levels were only slightly above the normal range when delivery occurred. So, prenatal treatment improves the fetal condition and the maternal disease as well (**Nyhan, 1975**).

**Thyrotoxicosis**

Fetal thyrotoxicosis is usually seen in infants of mothers with Grave disease or autoimmune thyroiditis. The diagnosis is made with cordo-centesis via assaying T4 level. Maternal treatment with Propyl-thiouracil (initial dose 300 mg/d PO, then titrate according to effect) or Methimazole is associated with a good fetal outcome.

**Hypothyroidism**

Fetal hypothyroidism is linked to maternal hyperthyroidism, use of radioactive iodine, drugs, and excessive maternal iodine intake. Fetal status is evaluated by ultrasonography and by direct cordocentesis via assaying reverse T3 level. Intra-amniotic L-Thyroxine (500 mcg q2wk initiated at 34 weeks of gestation) has been shown to cause regression of fetal goiters and normalization of hormone levels (**Bajoria et al., 1997**).

**Multiple carboxylase deficiency**

Biotin-responsive multiple carboxylase deficiency is an inborn error of metabolism due to diminished activity of the mitochondrial biotin-dependent enzymes: pyruvate carboxylase, propionyl-CoA carboxylase and B-methylcrotonyl CoA carboxylase. Affected patients present as newborns or in the early childhood period with dermatitis, severe metabolic acidosis, and a characteristic pattern of organic acid excretion. Metabolic alterations in affected patients can be restored toward normal with biotin supplementation.

Maternal prenatal administration of Biotin (10 mg per day) is therapeutic and results in delivery of homozygous neonates having no clinical or gross chemical abnormalities of the disorder (**Roth et al., 1982 & Packman et al, 1982**)

**Neural tube defects**

Maternal intake of Folic acid all through the peri-conception period (before conception to at least 1 month coinciding with completion of development of the neural tube in the fetus) has been proven to reduce the incidence of neural tube defects in women with one or more previously affected children and in women who have no risk factors.

Owing to its vital role in embryogenesis, all women are advised to take prophylactic folic acid supplementation prior to conception (0.5 mg/d for 3 months). Therapeutic folic acid supplementation (5 mg/d) is recommended for women with a previously affected child or with positive family history of neural tube defects, beginning at least 1 month prior to conception through 3 months of pregnancy (**Centers for disease control, 1992**).

**Lung immaturity**

Preterm delivery is a major cause of peri-natal morbidity and mortality, due to many factors headed by lung immaturity (**Avery and Mead, 1959**). In multiple studies, maternal corticosteroid therapy, used to induce lung maturity and surfactant synthesis in the fetus, has been proven effective in significantly reducing respiratory distress syndrome (RDS) in the neonatal period. Controlled studies have shown a reduction from 20.2% to 11.2%. Betamethasone (12 mg IM q24h for 2 doses) or Dexamethasone (6 mg IM q12h for 4 doses) is recommended for fetuses at 24-34 weeks' gestation that are at risk of preterm delivery (**Crowly et al., 1990**).

**Fetal Cardiac Therapy**

Most fetal arrhythmias are benign, and 90% are atrial extra systoles. These should be observed twice weekly to exclude sustained supra-ventricular extra systoles or atrial flutter. In ventricular extra systoles, myocardial ischemia and tumors, e.g. rhabdomyomas, must be excluded.

**Congenital heart disease**

The precise diagnosis of congenital heart lesions with the aid of newer echocardiographic techniques has created the potential for prenatal surgery or interventional catheterization.

In the treatment of hypoplastic left heart syndrome, umbilical vessel catheterization and balloon valvoplasty in utero for aortic stenosis are being attempted with equivocal results. In critical pulmonary stenosis, experimental valvotomy in utero may prevent development and or progression of hypoplasia of the right ventricle. At present, the major goal of prenatal diagnosis of congenital heart lesions is genetic counseling and delivery at a tertiary center, where early and optimal management is possible in the neonatal period (**Mahle et al., 2001**).

**2. Fetal surgery**

Most disorders diagnosed before birth are usually managed by treatment after birth. However, a few diseases with predictable devastating developmental consequences may benefit from intra-uterine fetal treatment. Surgical intervention on the human fetus has become an established therapeutic endeavor of many fetal malformations. Until recently, only fetuses with life threatening defects were considered candidates for prenatal correction, nowadays fetal surgical procedures are being performed for non-lethal conditions.

In spite of advances in this respect, ethical considerations are still a matter of much debate. The fundamental conflict in fetal surgery is balancing the risks to both mother and fetus against the potential benefit to only the fetus (**Flake, 2001**). As the mother is a bystander in the endeavor, her involvement entails only risk. A major intervention to save the life of a fetus seems warranted, and indicated, only if maternal risks can be minimized and good fetal outcome assured. If one can minimize maternal risk by avoiding hysterotomy and its attendant lifelong

risk of uterine rupture, then a more minor improvement in fetal outcome might be tolerated (**Lyerly, 2001 & Farrell, 1999**).

Principles developed in the 1980s that underlie clinical application of fetal surgery remain largely unchanged : documentation of the natural history of the untreated disease in-utero before clinical application, sound pathophysiological rationale for treatment before birth, demonstration of safety and efficacy of the fetal procedure in an appropriate animal model and development of inclusion and exclusion selection criteria for treatment. Rigorous groundwork has been accomplished for several anomalies that are amenable to fetal surgical intervention.

**Congenital diaphragmatic hernia**

Congenital diaphragmatic hernia (**CDH**) results from a defect in the development of the fetal diaphragm, in which the herniated abdominal viscera compress the growing fetal lung and prevent normal growth. The trends in treatment of CDH mirror the trends in all fetal surgery namely the trend from open hysterotomy to minimally invasive fetoscopic repair.

The procedure entails complete anatomic repair of the diaphragmatic defect after a maternal hysterotomy and partial removal of the fetus. An alternative strategy was developed to occlude the trachea temporarily leading to distending the hypoplastic lungs. This fetoscopic approach uses small scopes and video equipment, for detachable balloon placement by fetal bronchoscopy. An obvious major advantage of using video fetoscopic technology (**FETENDO**) surgery was the lack of maternal hysterotomy and its associated morbidity. Only fetuses most severely affected with liver herniated into the chest, diagnosis before 24 weeks, and a ratio of lung to head of less than 1.4 are eligible for treatment. Less severely affected fetuses are best managed after birth (**Harrison et al, 2001**).

**Obstructive uropathy**

Anomalies of the fetal urinary tract, most commonly posterior urethral valves, usually lead to oligo-hydramnios, pulmonary hypoplasia and even death. Fetuses with posterior urethral valves have been treated by drainage of the obstructed urinary system and restoration of amniotic fluid to normal levels. This was initially accomplished by open fetal surgery with vesico-amniotic shunting and is now done by inserting percutaneous vesico-amniotic shunts in a clinical office based setting. Careful selection of patients and timing are necessary to avoid salvaging fetuses whose lungs are functional but who go on to develop renal failure (**Walsh and Johnson, 1999**).

**Congenital cystic adenomatoid malformation**

Most fetuses diagnosed with a lung mass turn to have congenital cystic adenomatoid malformation which either undergoes spontaneous resolution or is best managed with close surveillance and treatment after birth. A small subset of fetuses with large lung lesions will become hydropic, deteriorate rapidly and die in utero. For this subset, open fetal surgical resection of the lung mass, involving maternal hysterotomy, fetal thoracotomy and ligation and resection of the mass has proved successful with a survival rate of over 60% (**Adzich et al., 1998**).

**Sacrococcygeal teratoma**

As with congenital cystic adenomatoid malformation, some fetuses with sacro-coccygeal teratomas are susceptible to demise in-utero if the vasculature of the tumour grows to a tremendous size and results in hydrops from high output cardiac failure (**Sbragia et al., 1998**). Maternal hysterotomy and resection of the tumor can save these fetuses. Recently, effectively stopping the blood flow to the tumor via percutaneous radiofrequency ablation has shown promise in treatment of the condition although accidental injury to adjacent soft tissues is a major risk of the procedure (**Paek et al., 2001**).

**Twin-twin transfusion syndrome**

Twin-twin transfusion syndrome is a devastating complication of monochorionic twin pregnancies, in which placental vascular connections result in one twin stealing the blood supply to the other twin, ultimately resulting in the death of both twins. Mortality of twin-twin transfusion syndrome diagnosed in the mid-trimester is greater than 80%. Fetoscopic laser ablation of abnormal placental vessels to treat this disorder has been tried and might prove to be a successful intervention modality for this anomaly (**Mari et al., 2001**).

**Myelomeningocele**

Spina bifida is a devastating malformation. Prenatal treatment of myelomeningocele via in-utero surgery to repair the spinal defect has been practiced repeatedly but no fetus has been cured of the disease. Published reports indicate that most fetuses show no appreciable improvement in their level of paralysis compared with that seen after optimum postnatal care However, as many as a third of the fetuses with the Arnold-Chiari malformation show improvement, thus decreasing the need for ventriculo-peritoneal shunting (**Cochrane et al., 2001**).

Fetal surgery for this disorder has been associated with serious maternal and fetal complications, including uterine rupture, maternal bleeding, fetal death and prematurity. In addition, the long term outcome for these patients is largely unknown as follow up for most of these fetuses has been poor. Many women elect termination of pregnancy and others choose natural birth and postnatal care for their afflicted fetuses. For women who wish to

consider treatment before birth, a multicentre randomized controlled trial of fetal surgery for myelomeningocele sponsored by the National Institutes of Health is under way. The results of this trial are critical to establish both the safety and the efficacy of this procedure before it becomes a recognized treatment approach.

## 7- Gene therapy

As the term implies, gene therapy aims at offering radical treatment of genetic diseases via correcting the underlying pathogenetic mechanisms of these diseases at the gene level. Based on our knowledge of these mechanisms, a wide variety of techniques have been theorized and experimented with varying success. unfortunately, quite few of them are worthy of trial. With the exception of the success of classic viral-based gene therapy for **superficial malignant melanoma** of the skin (**Maria Sotomayor et al., 2002)** and the success of stem cell therapy for some selected genetic disorders, the way to safe and successful gene therapy is still very long.

**Gene therapy techniques**

Gene therapy techniques for correcting defective genes responsible for disease development include several approaches:

1. A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene.
2. An abnormal gene could be swapped for a normal gene through homologous recombination.
3. The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.
4. The gene expression status of a particular gene could be altered via regulatory procedures.

Current trials of gene therapy techniques comprise many different approaches. Some of them target the deficient or defective gene function by trying to compensate for the lost function by offering normal counterparts of the diseased gene to affected cells or tissues through e.g. **gene transfer mechanisms.** Other techniques target the whole affected cells by offering normal cells with normal whole genomes, like the case in **stem cell therapy**. In between these two extremes of the spectrum, too many other techniques are being tested with hopeful expectations. These approaches aim at targeting underlying pathogenetic mechanisms at all possible stages, for example by manipulation of post-transcriptional effector molecules like mRNA by micro- or small- RNAs, manipulation of the translation machine in the cytosol by signal transducers or by manipulating post-translational events by chaperones. Such techniques include, for instance, the use of the ability of **hammerhead ribozyme** to induce site-specific cleavage of RNA to down-regulate the expression of mutant alleles (**Phylactou et al., 1998), the** use of **RNA interference** technology (iRNA) for control of underlying defects in movement disorders (**Hideki Mochizuki et al., 2008),** the use of nanoparticles, instead of viral vectors, as gene carriers to target cells for many diseases, e.g. lung cancer where nanoparticles are used to target cancer cells with dual tumor suppressor genes (**Jack Roth and Lin, 2007),** the induction of **molecular chimerism** as a potent regulator of cell functions to alter cell behavior e.g. induction of B-cell and T-cell tolerance for preventing graft rejection and prolonging survival of transplanted organs **(Iacomini and Bracy, 2001 & Bracy et al, 2001)** and use of **pharmaceutical molecular chaperones** to correct post-translational modifications defects which underlie the pathogenesis of a considerable number of common and serious genetic diseases like Alzheimer's disease, Parkinson's disease, Huntington's disease, Creutzfeldt–Jakob disease, cystic fibrosis, Gaucher disease and many other degenerative and neurodegenerative disorders (**Tapan and Subhankar, 2006**).

In most gene therapy studies, a correct copy or wild type gene is provided or inserted into the genome by the use of both viral and non-viral gene delivery systems. Generally, it is not an exact replacement of the abnormal disease-causing gene, but rather extra, correct copies of genes are provided to complement the loss of function. A carrier called a vector must be used to deliver the therapeutic gene to the patient's target cells. Currently, the most common type of vectors is viruses that have been genetically altered to carry normal human DNA. Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Scientists have tried to harness this ability by manipulating the viral genome to remove disease-causing genes and insert therapeutic ones (**Cavazzan et al., 2004**).

Target cells such as the patient's liver or lung cells are infected with the vector. The vector then unloads its genetic material containing the therapeutic human gene into the target cell. The generation of a functional protein product from the therapeutic gene restores the target diseased cell to a normal cell (**Gardlick et al., 2005**).

Substantial progress has been made in making gene transfer vehicles more efficient, less toxic, and non-immunogenic and in allowing long-term expression of the transferred gene. One of the key issues in successfully implementing gene therapies in the clinical setting is to be able to regulate gene expression very tightly and consistently as and when it is needed. The regulation ought to be achievable using a compound that should be nontoxic, be able to penetrate into the desired target tissue or organ, and have a half-life of a few hours (as opposed to minutes or days) so that when withdrawn or added (depending on the regulatable system used) gene expression can be turned on or off quickly and effectively. Also, the genetic switches employed should ideally be

non-immunogenic in the host. The ability to switch transgenes on and off would be of paramount importance not only when the therapy is no longer needed, but also in the case of the development of adverse side effects to the therapy (**Cavazzan et al., 2004**).

**Delivery system available for gene therapy**

The major aim of classic gene therapy is to introduce therapeutic genes into target cells, leading to efficient and stable expression of the therapeutic molecules and minimizing any putative adverse inflammatory or cytotoxic side effects. This can be achieved using viral and non-viral vectors. Important parameters to be considered when choosing a gene therapy vector include: (1) size limitations for insertion of transgenes (2) purity and titer of the vector (3) transduction efficiency (4) ability to infect dividing and/or quiescent cells (5) long-term expression of transgenes (6) integration into the host genome (7) the need for cell-type specificity or targeted delivery and (8) vector-associated toxicity and immunogenicity.

**Challenges to gene therapy techniques**

There are, still, a number of technical and non-technical challenges that have to be surpassed in order to formulate a safe, effective, consistent and permanent gene therapy for genetic disorders. Some of these challenges therapy include:

**1. Problems related to use of viral vectors**

viruses, while the carrier of choice in most gene therapy studies, present a variety of potential problems to the patient including pathogenicity, toxicity, immune and inflammatory responses, targeting and gene expression control issues. In addition, there is always the fear that the viral vector, once inside the patient, may recover its ability to cause disease. The potential for an oncogenic event to occur as a result of the random insertion of the gene into the host cell chromosomes is always a possible risk in these viral-based gene therapy trials (**Horn et al., 2004**).

**2. Short-lived nature of transferred genes**

Before gene therapy can become a permanent cure for any condition, the therapeutic gene introduced into target cells must remain functional and the cells containing it must be long-lived and stable. Also, there is the need to introduce the gene into a large number of cells in order to have a clinical effect. Problems with integrating therapeutic DNA into the genome and the rapidly dividing nature of many cells prevent gene therapy from achieving any long-term benefits. Patients will have to undergo multiple rounds of gene therapy trials to get acceptable benefit (**Horn et al., 2004**).

**1. Immunogenic responses**

The risk of stimulating the immune system in a way that reduces gene therapy effectiveness is always a possibility. Furthermore, the immune system's enhanced response to invaders it has seen before makes it difficult for gene therapy to be repeated in patients.

**4. Polygenic and Multifactorial disorders**

Conditions or disorders that arise from mutations in a single gene are the best candidates for gene therapy. Unfortunately, some of the most commonly occurring disorders, such as heart disease, high blood pressure, Alzheimer disease, arthritis, and diabetes, are caused by the combined effects of mutations involving many genes and many environmental triggers. Polygenic and Multifactorial disorders
would be especially difficult to treat effectively using conventional gene therapy.

**5. Risk of insertional mutagenesis**

If a transferred gene is integrated in the wrong place in the genome, for example in a tumor suppressor gene, it could induce a tumor. Clinical gene therapy trials using hematopoietic stem cells, transduced with a corrective transgene using a retrovirus, for treatment of X-linked severe combined immunodeficiency (X-SCID) led to the development of T cell leukemia in a number of treated patients (**Woods et al., 2006**).

**6. Gene delivery to inaccessible sites or non-dividing cells**

the difficulty of creating effective vectors, especially for gene delivery to inaccessible or non-dividing cells such as those in the brain is a real obstacle to invention of suitable approaches aiming at treating genetic disorders of brain cells. The same also applies to trials aiming at use of stem cells in this respect.

**Non-viral vector gene delivery systems**

In trying to circumvent safety issues inherent to the use of viral vectors, development of numerous non-viral vectors is actively being pursued. The underlying principle of non-viral vector systems is to complex DNA that carries a therapeutic gene with molecules that will facilitate DNA entry into the cells of interest. Complexed DNA binds to the cell membrane, triggering either non-specific or receptor-mediated endocytosis. Upon entry into the cell these complexes are contained in endosomes. The ability of these complexes to escape from endosomes before lysosomal enzymes destroy them is an essential characteristic of a successful non-viral vector. Once released from the endosomes, these complexes must enter the nucleus to undergo transcription. To be successfully transcribed, complexed DNA must be released from its carrier molecules and stably express RNA.

There are several types of non-viral vector systems being explored to find optimal carrier systems. Although uncomplexed DNA has been successfully used to transfect skeletal muscle (**Wolff, 1990**), systemic administration has been unsuccessful due to clearance of DNA by serum nucleases (**Niven et al., 1998** and **Houk et al., 1999**). The majority of non-viral vector systems use cationic lipids, polymers, or both as carriers. In these systems negatively charged DNA is strongly attracted to the lipid or polymer, and this interaction causes condensation of the DNA (**Golan et al., 1999**). Cationic lipids, which self-assemble into vesicles that adsorb DNA, are known as lipoplexes. The stability of these lipoplexes is dependent on the lipid/DNA ratio (**Schatzlein, 2001**). Since lipoplexes are electrostatically, but nonspecifically, attracted to cellular membranes, the specificity of this carrier is low. Polymers like poly-L-lysine (PLL) and polyethylenimine (PEI) have also been used in vitro to introduce DNA more efficiently into cells. The PLL/DNA ratio is essential for successful condensation and efficient intracellular release from endosomes. However, the use of PLL as a carrier agent is limited due to its toxicity (**Schatzlein, 2001**).

A major advantage of non-viral vectors, aside from avoiding the issues of viral safety, is that they remain episomal, allowing long-term and also high levels of gene expression. Success of this approach has been observed for trials aiming at targeting specific cells in many disease processes like muscle cells in Duchenne muscle dystrophy (**Gillis, 2004**), glial cells and primary neurons in neurodegeneration (**da Cruz et al., 2004**), fibroblasts in lysosomal storage disorders (**Estruch et al, 2001**), glial cells in cerebral ischemic diseases (**Wang et al., 2000**) and glioblastoma cells (**Hsiao et al., 1997**).

**Regulatory gene expression systems for gene therapy application**

Success of gene therapy hinges on several factors including site, duration, and levels of gene expression. Regulatory systems have been developed to control the temporal expression of a target gene in vitro and in vivo. Currently, the tetracycline regulatory system (**Gossen and Bujard, 1992**) is the most widely used and versatile system. Regulatory gene expression systems are an attractive development, and potential applications have been assessed in a wide variety of preclinical laboratory models of disease as in cancer, diabetes, arthritis, and ischemia, that make use of inducible gene expression systems.

**Current trials of gene therapy**

**1. Parkinson disease**

The first trial utilizing an adeno-associated virus (AAV) for treatment of Parkinson disease has recently been approved (**Staff, 2002**).

**2. Myocardial ischemia**

Inducible gene therapy vectors have proven to be very successful in preclinical models for treatment of myocardial ischemia. Although no clinical trials are yet under way, the vector system utilizes a hypoxia-sensitive promoter to regulate gene expression, and affords us a glimpse of an exciting future in gene therapy in which inducible vectors may lie dormant for months or even years before therapeutic gene expression is switched on in response to dangerous signals such as hypoxia (**Tang et al., 2002**).

**3. Diabetes mellitus**

Inducible insulin expression by gene therapeutic vectors would circumvent many of the disadvantages of conventional insulin therapy. Many researches have investigated the ability to engineer hepatocytes to express and secrete a functional modified insulin protein (**Auricchio et al., 2002**). Clinical trials in humans with diabetes unfortunately face many technical challenges that must be overcome first, including correct processing of the insulin transgene and stable expression of an inducible system that must be switched on and off many thousands of times (**Alam and Sollinger, 2002**).

**4. Leber's congenital amaurosis**

A safe, sub retinal delivery of recombinant adeno associated virus (AAV) carrying RPE65 gene has been tried for patients with this disease with alleged success. Treated patients are claimed to have modest increase in vision, and, perhaps more importantly, no apparent side-effects (**Maguire et al., 2008**).

**5. Organ transplantation**

One major complication facing organ transplant recipients is the requirement for life-long systemic immunosuppression to prevent rejection, which is associated with an increased incidence of malignancy and susceptibility to opportunistic infections. Gene therapy has the potential to eliminate problems associated with immunosuppression by allowing the production of immune modulatory proteins in the donor grafts resulting in local rather than systemic immunosuppression. Alternatively, gene therapy approaches could eliminate the requirement for general immunosuppression by allowing the induction of donor-specific tolerance. Gene therapy interventions may also be able to prevent graft damage owing to non-immune-mediated graft loss or injury and prevent chronic rejection (**Bagley and Iacomini, 2003).**

**6. Malignant tumors**

Gene therapy trials for treatment of cancer represent, probably, the major portion of research experimentation as well as the major contribution to advances in gene therapy techniques. A countless number of such techniques have been, and still being continually, designed aiming at targeting nearly all known pathogenetic mechanisms underlying development and progression of various aspects of the malignant phenotypes.

The three conventional modalities of treatment of cancer – surgery, radiotherapy and chemotherapy are often unsuccessful in treating cancer. Gene therapy is the emerging fourth modality for treatment of cancer. It can be used either alone or as an adjuvant to other treatment modalities. Certain genes can sensitize tumor cells to radiation or drugs and hence can be used to enhance the effect of the treatment. Gene therapy can also be used to debulk tumors which can then be removed by surgery. Various approaches are being examined in clinical trials for gene therapy for cancer, some of which are cited here.

**I. Targeting genetic lesions in tumor cells by antisense molecules**

Antisense molecules are synthetic oligo-deoxy-nucleotides (ODN) which are designed such that they can hybridize specifically to the coding (sense) mRNA inside the cell. Targeting mRNA with ODNs is attractive as they form Watson-Crick base pairs with the targeted mRNA. The double stranded RNA cannot be translated and is easily destroyed with consequent arrest of synthesis of oncoproteins that mediate tumor development (**Umberto Galderisi et al., 1999**).

**II. Immunomodulation by gene therapy**

Cancer patients generally have lowered immune response which can be augmented by gene therapy. It is now possible to genetically alter immune cells to increase their function. Therapeutic genes can be introduced ex-vivo either into the tumor cells or into the effector cells such as T lymphocytes or antigen presenting dendritic cells, or even to proximal or distant organ sites in the patient. Such a strategy can be used in combination with other strategies or even with any conventional modality of treatment (**Satoh Takefumi et al., 2004**).

**III. Genetically modified tumor vaccines in gene therapy**

Tumor cells as well as immune effector cells have been modified by insertion of various genes mainly cytokine and growth factor genes. Cytokines, which are small polypeptides involved in immunity and inflammation, are being extensively used in immunotherapy. Genetically modified tumor cells releasing various cytokines have been shown to result in local recruitment of inflammatory cells that in turn can inhibit tumor growth. This is accompanied by tumor antigen priming of the host immune system and enhanced tumor immunogenicity resulting in tumor regression. In animal studies, in some instances immunological memory has been generated to resist subsequent challenge with unmodified parental tumor cells. In a selected set of advanced cancer patients it has been demonstrated that high dose of the cytokine, interleukin-2 (IL-2), results in modification of the host immune system leading to tumor regression (**Kikuchi et al., 2000).**

**IV. Induction of apoptosis**

One of the major problems in treating solid tumors by either radiation therapy or chemotherapy is that the tumor cells are often resistant to apoptosis and therefore do not succumb to the conventional treatment. Hence, many therapeutic approaches have been aimed at killing cancer cells by inducing apoptosis. At the molecular level, mutation of the p53 tumor-suppressor gene is found in greater than 50% of human tumours. p53 plays a major role as a gatekeeper by inducing apoptosis in cells carrying damaged DNA. Wild type p53 has been shown to induce apoptosis in squamous cell carcinoma cell lines and has also been used in phase-1 trials of adenoviral-p53 transfer in patients with advanced squamous cell carcinoma of head and neck in a surgical adjuvant setting. Wild type p53 has been used either alone or in combination with other apoptosis-inducing genes, or in combination with radiotherapy. Overexpression of pro-apoptotic molecules such as Bax favor death of cells resistant to ionizing radiation. Expression of Bax could sensitize radio-refractory cells to radiotherapy. Caspase-8, a member of the family of Caspases, is also involved in bringing about apoptosis. Preclinical studies have indicated that caspase-8 effectively induced cell death in gliomas and could be a useful strategy for gene therapy of gliomas (**Stacie Bianco et al., 2003**).

**V. Blocking angiogenesis**

Tumors require a constant supply of oxygen, nutrients, hormones and growth factors for their existence and dissemination. This is provided by formation of new blood vessels or angiogenesis. Experimental tumors have been shown to regress by inhibiting angiogenesis and this has made it a suitable target for gene therapy. Two popular inhibitors of angiogenesis are angiostatin and endostatin. These are naturally generated by proteolysis of larger proteins such as plasminogen (for angiostatin) and collagen XVIII (for endostatin). In phase I clinical trials with human recombinant endostatin no dose-limiting toxicity was observed. However, for continuous administration of the protein gene therapy approaches are preferred. Genes coding for the angiogenesis inhibitors can be introduced either directly into the patient's cells ex vivo or through generic cells that have been genetically modified to overexpress the protein of interest. In order to protect the generic cells from immunological destruction, two groups have made anti-angiogenic cell factories embedded in alginate beads and implanted them

in animal models for brain tumours. Both the groups have shown considerable reduction in tumour growth (**Xiaojun Huang et al., 2001**).

**Current status of gene therapy research**

The Food and Drug Administration (FDA) has not yet approved any human gene therapy product for sale. Current gene therapy is experimental and has not proven very successful in clinical trials. Little progress has been made since the first gene therapy clinical trial began in 1990. In 1999, gene therapy suffered a major setback with the death of 18-year-old patient who was participating in a gene therapy trial for ornithine trans-carboxylase deficiency (OTCD). The patient expired from multiple organ failures 4 days after starting the treatment. His death is believed to have been triggered by a severe immune response to the adenovirus carrier.

Another major blow came in January 2003, when the FDA placed a temporary halt on all gene therapy trials using retroviral vectors in blood stem cells. FDA took this action after a second child treated in a gene therapy trial had developed a leukemia-like condition. Both this child and another who had developed a similar condition in August 2002 had been successfully treated by gene therapy for X-linked severe combined immunodeficiency disease (X-SCID).

FDA's Biological Response Modifiers Advisory Committee (BRMAC) met at the end of February 2003 to discuss possible measures that could allow a number of retroviral gene therapy trials for treatment of life-threatening diseases to proceed with appropriate safeguards. In April of 2003 the FDA eased the ban on gene therapy trials using retroviral vectors in blood stem cells.

## 8- Miscellaneous topics

**A. Therapeutic approaches to mitochondrial disorders**

Treatment options in primary mitochondrial disorders are limited and of doubtful effectiveness, there is no established treatment and current management is largely supportive. General measures for management of these disorders include the following:

1. Adequate intake of energy, fluids and electrolytes.
2. Restriction of glucose intake and addition of lipid supplements (1-2 g/kg/day) if fatty acid oxidation defects are excluded.
3. Immediate management of conditions that encroaches on energy consumption particularly fever and seizures.
4. Avoidance of drugs and medications that may inhibit the respiratory chain enzymatic and metabolic pathways like Valproate, Tetracyclines and Chloramphenicol.
5. L-Carnitine supplementation (50-100 mg/kg/day) after exclusion of fatty acid oxidation defects and assaying Carnitine blood level.
6. Treatment of acidosis by Sodium bicarbonate.
7. Vitamins and cofactors supplementation to support mitochondrial enzymes like: Idebenone or coenzyme Q10 (5-10 mg/kg/day), Biotin (20 mg/day), Creatine (100-200 mg/kg/day) in isolated mitochondrial myopathy, and ketogenic diet and Thiamine (500-2000 mg/day) in Pyruvate dehydrogenase complex deficiency (**Zschocke and Hoffmann, 1999**).

**New trends and prospects in treatment of Mitochondrial disorders**

1. Recent advances in our understanding of the pathophysiology of mitochondrial diseases provide hope for novel treatments. Patients with mitochondrial myopathy due to mutations of mitochondrial DNA (mtDNA) may benefit from treatments that move normal mitochondrial genomes from the muscle satellite cells into skeletal muscle, but there are concerns about the long-term effects of this approach. A greater understanding of the pathophysiology of a number of nuclear genetic mitochondrial disorders suggests new avenues for treatment (such as copper-histidine in children with SCO2 gene mutations, and strategies modifying intra-mitochondrial nucleoside pools in the various disorders of mtDNA maintenance). A number of different strategies are also being explored at the molecular level, including the **use of antigenomic molecules to mutated mtDNA** and the allotropic expression of mutated mtDNA genes within the cell nucleus. Nuclear transfer techniques also provide hope for women at risk of transmitting pathogenic mtDNA mutations (**Chinnery, 2004**).
2. Although therapy of mitochondrial encephalo-myopathies is woefully inadequate despite great progress in our understanding of the molecular bases of these disorders, palliative measures are dictated by good medical practice and includes anticonvulsant medication, control of endocrine dysfunction, and surgical procedures. Removal of noxious metabolites is centered on combating lactic acidosis, but extends to other metabolites. Attempts to bypass blocks in the respiratory chain by administration of electron acceptors have not been successful, but this may be amenable to genetic engineering. Administration of metabolites and cofactors is the mainstay of real-life therapy and is especially important in disorders due to primary deficiencies of specific compounds, such as carnitine or coenzyme Q10.

There is increasing interest in the administration of **reactive oxygen species scavengers** both in primary mitochondrial diseases and in neurodegenerative diseases directly or indirectly related to mitochondrial

dysfunction. Aerobic exercise and physical therapy prevent or correct deconditioning and improve exercise tolerance in patients with mitochondrial myopathies due to mitochondrial DNA (mtDNA) mutations. Gene therapy is a challenge because of polyplasmy and heteroplasmy, but interesting experimental approaches are being pursued and include, for example, decreasing the ratio of mutant to wild-type mitochondrial genomes (**gene shifting**), converting mutated mtDNA genes into normal nuclear DNA genes (**allotopic expression**), importing cognate genes from other species, or correcting mtDNA mutations with specific restriction endonucleases.

Germline therapy raises ethical problems but is being considered for prevention of maternal transmission of mtDNA mutations. Preventive therapy through genetic counseling and prenatal diagnosis is becoming increasingly important for nuclear DNA-related disorders. Progress in each of these approaches provides some glimmer of hope for the future, although much work remains to be done (**DiMauro and Mancuso, 2007**).

**B. Therapeutic approaches to lysosomal storage and allied diseases**

There are several major therapeutic approaches that may ultimately be useful in the treatment of the lysosomal storage and allied diseases. The majority of these approaches, enzyme replacement therapy; gene therapy; bone marrow transplantation; neural stem cell therapy and molecular or pharmacological chaperone therapy, aim at restoring enzyme activity. Other interventions, substrate deprivation and metabolic bypass therapy, are not aimed at restoring enzyme activity but are aimed at the reduction in the levels of the compounds that accumulate in the lysosomes. These are currently more theoretical approaches to therapy and researches still have much to explore and achieve in this regard. The most difficult diseases to treat are those which affect the central nervous system, obviously, no single approach will be the solution for any one of these devastating diseases. Instead, the hope is that some combination of these approaches - will be able to halt, or even reverse, the ravaging effects of diseases like Tay-Sachs disease, Canavan disease, Sandhoff disease and others that ravage the brain.

**1. Bone marrow or stem cell transplantation** makes use of the fact that certain brain cells, as well as blood cells, arise from bone marrow or stem cells isolated from umbilical cord blood. Researchers theorize that such transplantation from a healthy donor would introduce healthy stem cells into the brain and multiply. However, it is not known if the number of healthy cells would be large enough to provide enough of the missing enzyme to make a clinical difference. If not, healthy stem cells could be genetically manipulated prior to transplantation to increase the production of the missing enzyme. Other factors which could limit the potential of bone marrow transplantation for the treatment of the lysosomal storage and allied diseases include the need to find an immunologically matched healthy bone marrow donor and the risks associated with the bone marrow transplantation procedure. However finding a match with cryopreserved fetal cord blood is much easier and the risks associated with the transplant procedure are somewhat less than with conventional bone marrow transplantation.

**2. Stem cells** are immature cells that have the capability to develop into all of the different types of cells. The two leading sources of cells include mesenchymal cells (from blood) and neural stem cells (from brain). In mice, stem cells have been shown to spread throughout the brain, a necessity when it comes to treating diseases that affect the entire brain. Stem cells can also be genetically modified to produce and secrete high levels of a missing enzyme, such as Hex-A. Human clinical trials may still be many years away but the results in mice are promising. For example, when neural stem cells genetically engineered to produce Hex-A were inserted into the brains of mice with Tay-Sachs disease they traveled throughout the brain and produced therapeutic levels of the needed enzyme. The enzyme then broke down the GM2 ganglioside, preventing its accumulation with apparent clinical improvement.

**3. Gene therapy** is a revolutionary therapeutic breakthrough in the field of medical genetics and many other medical branches as well. It implies intervention in which the gene that is mutated in affected individuals is augmented by the introduction of a functional version of the gene. The gene can be introduced as free DNA, in a lipid coat (liposome) or as part of a viral vector. The latter is the most common way of introducing genes and it involves modification of a specific virus so that it cannot cause disease and then having it carry the gene for the missing enzyme to the brain or any other organ of interest. Gene therapy is under investigation as a treatment for numerous disorders, including Canavan disease **(Lin et al., 2007**).

**4. Metabolic bypass therapy** is another therapeutic biochemical approach that makes use of special chemicals called activators to increase the synthesis or activity of alternative lysosomal enzymes to make them capable of degrading larger amounts of a substrate like GM2 than normal. If one could increase the activity of an enzyme other than Hex-A to degrade GM2, then it could partially compensate for the absence of Hex-A and (by-pass) the cell's need for Hex-A. Inspite of preliminary promising successful results in animal studies, this approach is still in its beginning and researchers are hopefully looking for activators and appropriate (by-pass) enzymes for many allied disorders (**Chunmei et al., 2007**).

**5. Pharmacological or molecular chaperone therapy** is among the newest therapeutic ideas for storage and the allied diseases. As of summer 2006, pharmacological chaperone therapy is in early stage clinical trials for two lysosomal storage diseases; viz. Fabry disease and Gaucher Type I disease **(Gary Hin-Fai Yam et al, 2006).**

**Substrate deprivation approach** (also called substrate synthesis inhibition, substrate reduction, substrate balancing) is a biochemical approach that makes use of novel chemicals called inhibitors that decrease the production of the molecule that typically accumulates to high levels in persons with lysosomal storage diseases. For example, children with Tay-Sachs disease accumulate high levels of $G_{M2}$ in brain cells and it is this accumulation which causes the brain cells to die. If one could decrease the synthesis of GM2, the substrate for the missing enzyme, then one would presumably decrease cell death and moderate the course of the disease. The inhibitor Zavesca ® (miglustat), approved in Europe and the United States for the treatment of Gaucher Type 1, is in clinical trials in persons affected with Niemann-Pick Type C, and in children with juvenile $G_{M2}$, Tay-Sachs and Sandhoff and in children under the age of 2 with Tay-Sachs and Sandhoff disease. A three-year clinical trial of miglustat in persons affected with Late Onset Tay-Sachs disease ended in spring 2006, with unsatisfactory conclusions. Participants did not reach certain clinical endpoints that were part of the trial, such as improved muscle strength status, and Actelion, the drug company, decided not to file for an additional FDA indication for use with LOTS. Studies with other substrate reduction compounds may occur in the future, and some affected individuals or parents of those affected do explore with their physicians the option of using this treatment on an "off-label" basis **(Lachmann and Platt, 2001)**.

**Theoretical principle of substrate deprivation therapy (Trickle-Down Therapy)**

1. In most individuals the substrate can be degraded efficiently by adequate enzyme
2. In affected individuals the amount of enzyme is insufficient to degrade the accumulated substrate
3. In affected individuals treated with substrate synthesis inhibitors the amount of substrate is decreased to match the amount of residual enzyme to prevent accumulation.

## 9- Conclusions and recommendations

**I.** Genetic diseases are lifelong source of suffering to affected patients and their families. They represent a grievous and real burden on human health. Unfortunately, unlike most other disease categories, no radical cure could be offered to these patients. However, **a lot of alleviating and supportive therapeutic approaches have been, and continually being, formulated and designed** for these diseases with amazing wide spectrum having the simplest practice of diet restriction at one end and some of the most sophisticated gene therapy techniques, like mRNA interference measures, at the other end. New approved and documented advances in treatment and alleviation of genetic disorders are added continuously to the medical literature. Medical geneticists, and other care givers involved in management of these diseases, must always keep up following these advances in order to evaluate the potential and possibility of their safe and beneficial use in their patients.

**II.** Though proper diagnosis is a prerequisite of any therapeutic intervention, many treatment trials of many genetic disorders are based on mere theoretical assumptions extracted from our knowledge of the underlying pathogenetic mechanisms and pathophysiological alterations underlying the development and progression of these diseases. The desperate nature of most genetic defects might justify this non evidence-based medical practice. However, **if no appreciable improvement could be observed within a reasonable time of using these trials it is wise to stop them** and rely mostly on symptomatic supportive measures. This situation is met with and is recommended, for instance, in management of mitochondrial disorders which have no definitive therapy yet.

**III.** Thorough understanding and complete awareness of the whole aspect of drug function(s) is mandatory and of prime importance before drug prescription for genetic diseases. It must be always kept in mind that drug interactions, including conflicting effects, are the rule for most multidrug usage approaches: a drug might exert a required beneficial therapeutic effect by affecting one or more metabolic pathway with concomitant occurrence of unwanted, sometimes serious or fatal, side effects due to its effect on other pathways. Accordingly, drug therapy must be dealt with cautiously and preliminary and follow up investigations to predict possible side effects must be a part of routine drug therapy indicated for any genetic diseases. These guidelines particularly apply for cases requiring multidrug management which is quite common in genetic states due to the chronic lifelong nature of these errors. Drug interactions might cause serious and fatal sequels, and **strict drug management protocols for both single and multidrug therapy usage in these patients must be constructed and formulated and care givers must adhere to these protocols quite rigidly without any inattention**.

**IV.** In the vast majority of genetically-determined disease states, combined therapeutic approaches are needed often to accomplish the best therapeutic and prognostic results. According to the nature of each disorder, such combined approaches might synergize to counteract underlying pathogenetic mechanisms, to limit progressive damage caused by continuing exposure to genetic and/or environmental insults or by continued accumulation of toxic substrates or metabolic intermediate products, to combat resulting metabolic or pathophysiological alterations, to supply deficient gene products or other biomolecules, or to offer near radical cure in some conditions, e.g. via organ transplantation. So, **proper evaluation and selection of all necessary treatment strategies of a specific disease, in order not to ignore any of them, is of utmost importance as a first step in determining the management plan of the disease** in question.

**V.** The underlying pathogenetic mechanism(s) and the resulting pathophysiological alteration(s) of each disease determine the proper therapeutic approache(s) as well as the priority of each intervention modality. **Arrangement of these approaches in order of their beneficial effects on the patient** determines the guideline of their step-by-step or combined use in management.

**VI.** The role of **supportive intervention measures** can not, and should not, be ignored or overcome in management plans of genetic disorders. Such supportive measures include physiotherapy, speech therapy, prostheses, hearing aids, visual aids and orthopedic devices according to the nature of each disease. The same also applies for trends aiming at institution of **early intervention protocols** and **rehabilitation programs** for patients born with genetically-determined mental and/or physical handicaps, or patients who have lost the ability to function normally, often because of the progressive nature of their genetic disorder.

**VII.** The availability and overlap of many different treatment approaches to most genetic disorders necessitates **co-operative team approach** of different specialties in order to achieve the best therapeutic and/or prophylactic prospects of these patients. In addition to the medical geneticist, and according to the nature of each disease, the management team might include a biochemical geneticist, a specialized laboratory doctor, a dietitian specialized in dietetic management of metabolic disorders, a pharmacist familiar with drug/formula preparations commonly used in genetic diseases, a plastic surgery team experienced in surgical management of different types of congenital malformations and a psychiatrist specialized in management of dispriviliged patients and support of their desperate family members. This **concept of team work approach** in management of genetic disorders is crucial for success of therapy since it offers the best modalities and optimal conditions of medical, surgical and psychiatric care that can be availed and offered to the patient.

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